THE DESIGN AND MANAGEMENT OF TANKS FOR THE CULTURE OF TURBOT (*Scophthalmus maximus* (L.))

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ABSTRACT

The culture of turbot Scophthalmus maximus is currently conducted only in tanks, unlike many pelagic species which are also grown in net cages. Despite the demersal habit of this species, deep pelagic fish tanks are often used with little or no adaptations.

A multidisciplinary study was conducted to establish the consequences of several management regimes, primarily a decrease in water depth, on more than a total of 40 biological, water quality and hydrodynamic parameters. Fish fitness, performance and behaviour, exit age distribution, flow visualization, flow velocity determination and water quality determination studies were conducted.

Results were discussed in relation to optimal tank management strategies, suitable tank designs, or adaptations to existing designs. A statistical model was proposed. A decrease in water depth from 18 - 9 cm decreased dead volumes from 6.95 - 1.37 l. An increase in depth from 3 - 18 cm increased turbot specific growth rate by 0.44 % day^{-1}. Tank hydrodynamics had little influence on biological or water quality parameters, despite the large range of water depths relative to the size of the tanks. It was more probable that stocking density and biomass were the major influences on water quality and this in turn may have influenced fish performance. The advantages of reducing water depth in a culture tank were more numerous than the disadvantages.

GLOSSARY

ANOVA	=	analysis of variance
BOD	=	biological oxygen demand
CF	=	condition factor
COB	=	"Co-op blue food colour" dye tracer
COG	=	"Co-op green food colour" dye tracer
C_t	=	tracer concentration at time t (mg l^{-1})
DAFS	=	Department of Agriculture and Fisheries for Scotland
depth	=	unless otherwise stated refers to water depth, not tank depth (cm)
df	=	degrees of freedom
DO	=	dissolved oxygen (mg l^{-1} or % saturation)
d_t	=	change in time = time interval between samples
$E_{(t)}$	=	proportion of total tracer exiting tank at time t (min^{-1})
FCR	=	food conversion ratio, dry or wet weight of feed eaten: live fish weight increase
flow type	=	tanks with either the same residence time or the same flow rate
G_w	=	specific growth rate (% day^{-1})
H0	=	null hypothesis
HDPE	=	high density polyethylene
MAFF	=	Ministry of Agriculture, Fisheries and Food
NH_3-N	=	unionized ammonia nitrogen (concentration measured as ug NH_3-N l^{-1})
NH_4-N	=	total ammonia nitrogen (concentration measured as mg NH_4-N l^{-1})
NO_2-N	=	nitrite nitrogen (concentration measured as mg NO_2-N l^{-1})
p	=	level of significance (maximum acceptable limit: p = 0.05)

pigm.	=	fish pigmentation (wild or malpigmented)
Q_t	=	tracer quantity (g min l^{-1})
r	=	correlation coefficient
residence time	=	theoretical time taken for one complete change of water in a tank = t_i (min)
SCB	=	"Supercook blue food colouring" dye tracer
sd	=	dimensionless measure of the standard deviation of the distribution of the residence times
SD	=	stocking density (g m^{-2} or g m^{-3})
SDa	=	stocking density calculated as a factor of tank floor surface area (g m^{-2})
SDv	=	stocking density calculated as a factor of tank water volume (g m^{-3})
t_c	=	calculated mean residence time - actual (min)
t_i	=	ideal mean residence time
t_m	=	mean residence time (min)
V	=	tank water (or working) volume (l)
V_d	=	dead volume (l)
Vu	=	coefficient of variance
WDE1	=	water depth experiment 1 - Chapter 8
WDE2	=	water depth experiment 2 - Chapter 9
WFA	=	White Fish Authority
$\hat{E}C$	=	sum of tracer concentrations (mg l^{-1})
θ	=	dimensionless time
θ_c	=	ratio $t_c:t_i$
$\%V_d$	=	proportion of the total tank water volume which was a dead volume (%)

CHAPTER 1

INTRODUCTION

1.1 THE TURBOT

Wheeler (1969) describes the turbot, Scophthalmus maximus (L.) Bothidae, as an important food fish in European waters. It is an inshore flatfish found at depths to 80 m. Spawning occurs from April to August in the North and Irish Seas at depths between 10 - 80 m. Larvae, which hatch after 7 - 9 days are pelagic and have a swim-bladder until metamorphosis, when they become demersal at an age of 4 - 6 months. Adults, which are almost entirely piscivorous, prefer to inhabit a gravel substrate, though they are often caught on sand.

1.2 HISTORY OF FLATFISH AND TURBOT CULTIVATION AND RESEARCH

Initial investigations into the rearing of flatfish date from the beginning of the 20th century. Some early successes at rearing plaice Pleuronectes platessa (Dannevig, 1897), Dover sole Solea solea (Fabre-Domergue and Bietrix, 1905) and turbot (Anthony, 1908) were achieved. One of the earliest accounts of the cultivation of turbot (Anthony, 1908) indicated that the species would be profitable to farm due to a high market price, rapid growth and hardiness.

Despite this promising start it was not until the late 1950's that marine flatfish trials were resumed in earnest when, "significant quantities", of juvenile flatfish became available (Smith, 1979). The pioneering work which was carried out at Lowestoft by the Ministry of Agriculture, Fisheries and Food (MAFF) and at Port Erin, Isle of Man by the White Fish Authority (WFA) was described by Shelbourne (1976) and Kerr (1976).

Shelbourne *et al*. (1963) described the mass production of plaice eggs at Lowestoft between 1957 and 1960, though survival from the egg stage was only 3 - 5 %. Annual production at that time rose from 1 to 1200 fish (Shelbourne, 1963). Survival increases were primarily the result of improved water quality before 1960 and improved feeds and lighting in 1961 (Riley and Thacker, 1963). During long term plaice rearing experiments from 1962 onwards, survival rates exceeded 65 %, because of better temperature and lighting regimes, bacterial control and tank design (Shelbourne, 1970). Preliminary experiments with sole in 1965 proved unsuccessful and attempts to ranch flounders (*Platichthys flesus*) and plaice in an artificially enclosed sea loch at Ardtoe, West Scotland also failed due to deoxygenation, freshwater dilution and shore crab (*Carcinus maenas*) predation (Shelbourne, 1970).

Despite continued improvements in flatfish culture, Dover sole appeared too sensitive for intensive culture and plaice were considered unsuitable for cultivation

because of slow growth and low market price (Jones, 1972). Both of the two remaining most valuable species of flatfish, halibut (Hippoglossus hippoglossus) and turbot were difficult to rear during early work (Kerr, 1976). The first successful production of metamorphosed halibut, at Austevoll, near Bergen, Norway, was not achieved until 1985 (Dye and Brancker, 1987). Halibut rearing trials in this country are currently being carried out by the Sea Fish Industry Authority at Ardtoe, as described by Dye and Brancker (1987).

Jones (1972) analysed the factors which determined the profitability of a species for culture, ie. market price, growth rate, feeding level, food conversion, provision of stock and production rates. He concluded that turbot would be suitable for cultivation.

In 1970 - 1971, wild juvenile turbot were caught for study purposes. These were ongrown by MAFF using greatly adapted plaice husbandry techniques (Smith, 1979). Also during 1970, workers at MAFF first succeeded in hatching turbot, allowing further nursery and ongrowing work (Hull and Edwards, 1976). Preliminary culture suitability trials were conducted using both hatchery and wild stock turbot juveniles (Purdom et al., 1972; Purdom, 1973), during which females were found to grow faster than males after 19 months. The hatchery stock achieved 1.3 cm $month^{-1}$ growth, while wild stock gained 0.9 cm $month^{-1}$. It was concluded that the species had a high food

conversion rate of over 40 % at 18 °C and was easy to manage. No hierarchies developed and they were resistant to handling damage (Purdom et al., 1972).

Having, in principle, established the economic viability of turbot culture, work was carried out at Lowestoft and Port Erin into factors affecting the poor larval survival (Jones 1974; Jones et al., 1974), which reached only 10 % despite improvements to the culture system. Probable causes were inadequate tank design and management, dissolved oxygen concentration, food type and presentation (Jones et al., 1974). A nursery technique was developed by the WFA to produce young turbot at a mean weight greater than 50 g at the end of their first winter (Smith, 1979). During this nursery period and subsequent ongrowing, both onshore tanks and floating net cages were used (Howard and Kingwell, 1975; WFA, 1975b). Turbot grew to 240 g after a further 12 months, with a 90 % survival rate after metamorphosis (Hull and Edwards, 1976).

A further study of the commercial costs of farming turbot in 1975, again concluded that the species was economically viable to culture despite the incomplete development of many of the culture systems (WFA, 1975b). The improvements required included flow rate optimization, ongrowing density and tank culture husbandry costs.

Following this initial work, research has been conducted into three aspects of turbot culture relevant to

this study: improving hatchery efficiency (Olesen and Minck, 1983); nursery growth in onshore tanks (Kerr, 1976; WFA, 1977; 1979; Scherrer, 1984); and ongrowing in either onshore tanks (Kerr, 1976; Thain, 1979; Jones et al., 1980; Person-Le Ruyet, 1980; Poxton et al., 1982; Brown et al., 1984) or in floating net cages (Hull and Edwards, 1976; 1979; Linfoot and Hall, 1987).

1.3 THE NEED FOR FLATFISH SPECIFIC TANKS

It appears that two main problem areas have delayed the proliferation of the culture of turbot. Development of a suitable diet for turbot has been conducted by many workers. Bromley (1980b) looked at dietary water content; Adron et al. (1976), Bromley (1980a) and Caceres-Martinez et al. (1984) studied the effects of different levels of protein and energy; Leger et al. (1979) and Bell et al. (1985) examined the effects of dietary fatty acids; and Kuhlman et al. (1981), Deniel (1976) and Bromley and Howell (1983) tested new feed formulations or ingredients.

The other problem area is associated with ongrowing. At present, turbot eggs are hatched and the larvae are then weaned and grown through metamorphosis at specific hatchery facilities. Larval survival was low until recently, compared with other cultured species, but those juveniles which do survive are of a high quality and lead to reliable ongrowing results (Anon., 1987). These juveniles are ready for transfer to nursery facilities,

possibly at another site, at a weight of 2 g or less (Anon., 1987). A 70 % survival rate can be achieved if the juveniles are allowed to grow to more than 0.5 g before transfer to nursery facilities (Smith, 1979). At this stage, stock treatment varies according to the facilities available. The fish may be grown to marketable size at the same site as the hatchery, a method employed by Golden Sea Produce at Hunterston, Scotland until recently (pers. obs.). Alternatively they may be transferred to another site with adequate facilities and water temperature. This method is employed by Clearwater Aquaculture (International) Ltd, on the Isle of Man. The number of available sites for ongrowing is limited, particularly in the British Isles. Limitations include the availability of water temperatures approximating the optimal for growth of 19 ^{o}C (Jones et al., 1980), adequate onshore tank facilities or suitable net cages.

Marine salmonid ongrowing is primarily conducted in sea cages (Laird and Needham, 1988) so reducing the need for onshore pumped facilities. At present however a design of floating net cage for the successful culture of flatfish in general, and turbot in particular, is not available. Turbot are a demersal species and as such mainly lie on the substratum. Floating and semi-submerged net cages are moved by wave action and this causes oscillations at the floor of the cage, which may be stressful to the fish lying on it, particularly when the wavelength corresponds to the length of the collar and

cage (Linfoot and Hall, 1987).

Two possible culture strategies for ongrowing turbot in the future may develop, both of which require the use of tank facilities. If a feasible cage design for ongrowing turbot is produced, it is expected that the use of onshore facilities will be limited to the culture of hatchery and nursery stage fish. These will require a greater control of their feeding and environment than final fattening fish. In this situation turbot ongrowing will become available to companies with a small capital base. A situation, similar to that of the salmonid farming industry may prevail, in which a limited number of hatcheries provide juveniles for ongrowing at many farms. If the turbot culture industry adopts such a strategy, survival will be maximized if the hatchery or ongrowing farm can retain the juveniles until at least 0.5 g weight as suggested by Smith (1979), before transfer to juvenile tanks followed by ongrowing in either tanks or, possibly in the future, cages. Alternatively the current strategy is to ongrow the turbot in tanks. Either strategy requires extensive use of tanks for the culture of this species.

1.4 THE STUDY OF TURBOT TANK DESIGN

A major piece of aquaculture equipment which is often taken for granted is the holding structure or tank itself. Chapter 3 indicates that while tank design and management

have been considered important by numerous previous workers, very little work has been carried out to develop tanks for demersal species, eg. flatfish and some adult crustaceans, which require space as substrate area rather than volume. It was expected that the environmental requirements of a demersal species would differ from those of a pelagic species, and that responses to changes in facilities and environment would be different. Very little information regarding the culture facilities required by demersal species was however available.

Obvious adaptions to tank designs specific to demersal species include an increase in the floor surface area to water volume ratio by the use of internal structures such as shelves or layering, or a decrease in water level. The introduction of internal tank structures has however been shown by Surber (1936) to disrupt water flow patterns. Burley and Klapsis (1985) have indicated that changes in water depth have resulted in altered flows and mixing within tanks. Small changes in tank design or management, eg. inflow rate or direction are known to cause major changes in water movements (Wheaton, 1972). Water movements will in turn influence the distribution of: particulate waste, eg. faeces and uneaten pellets (Larmoyeux et al., 1973); dissolved nitrogenous metabolites (Rosenthal et al., 1982), eg. total ammonia and nitrite; and the introduction and distribution of substances required by the fish eg. feed and dissolved oxygen (Rosenthal et al., 1985). Poor water quality has

been shown to influence fish fitness and performance (see sections 8.5.8 - 8.5.13). A circumstantial, a priori link between tank hydrodynamics and the biology of the culture species has therefore been established, though little quantification of the inter-relationships between these parameters and water quality has been conducted. Chapter 3 indicates that few studies of fish tank design have incorporated aspects of water quality, biology and hydrodynamics. A notable exception to this was the classic paper by Burrows and Chenoweth (1955). Sections 5.1, 8.1 and 9.1 indicate how adaptions to tank management and design have influenced hydrodynamics, water quality and the biology of the culture species.

A study of tank design for a particular purpose may either investigate novel designs, or adaptions to existing designs. With the great and variable consequences of small scale changes in tank design and management which have been demonstrated, each tank may be considered unique, so specific adaptions to the design of one type of tank may not necessarily have the same effect on other designs. Currently only pelagic fish tanks are available from commercial tank suppliers and several turbot farming companies have utilized such tanks. It was therefore considered that the investigation which may be of most interest would be the determination and quantification of the effect of a general adaption to tank design and management, rather than specific designs, which would also indicate the underlying principles regarding the inter-

relationships between hydrodynamic, water quality and biological parameters. These principles could then be applied to many different designs of tank used for culturing turbot, to either optimize fish growth in a tank, or estimate the culture potential of a tank.

One such adaption to the management of a tank which would allow the principles of tank design to be studied was the reduction of water depth. This may be possible because turbot, as a demersal species, occupy only the lowest levels of a tank for the majority of the time, so upper levels are unoccupied. The expected consequences of such an adaption are discussed in section 8.1. This study therefore aimed to determine the influence of a range of water depths on water quality, tank hydrodynamics, fish performance and fitness. To achieve this aim, detailed studies were conducted of many different aspects of these parameters and their inter-relationships.

The following greatly summarized scheme was adopted. Detailed explanations of the experiments are indicated in the appropriate chapters.

Chapter 2: the objectives of the study were defined.

Chapter 3: a review of the relevant literature to determine both the most suitable designs of tanks and the principles which should be considered when designing or managing a tank.

Chapter 4: indicates the general techniques and materials which were used frequently during the study. Materials

and methods specific to a particular experiment have been detailed in the relevant chapters.

Chapter 5: was the first of three studies of the tank hydrodynamics. Adapting a standard process engineering technique, various aspects of mixing in the culture tanks, during different treatment regimes, were quantified.

Chapter 6: experiments were conducted to illustrate, rather than quantify, water movements within the tanks.

Chapter 7: was conducted to quantify any direct influence of one aspect of tank flow dynamics on the culture species, to aid the determination of the cause of any influence of water depth change on the fish, ie. either because of any water quality changes or directly due to hydrodynamics.

Chapter 8: was the first of two detailed studies of the influence of water depth on water quality, turbot fitness and performance. Changes in water depth altered the water volume, so it was not possible to maintain stocking density, calculated both as a factor of volume and of floor surface, constant at the same time. During this first experiment a constant stocking density, expressed in terms of surface area, was maintained.

Chapter 9: was a replicate experiment of that presented in Chapter 8, with the exception of stocking density, which was calculated in terms of water volume.

Chapter 10: draws together data from many different experiments in this study, to indicate certain underlying principles. Predictions of how alterations in one

parameter may influence others, were proposed using simple models.

Chapter 11: discusses overall conclusions from different parts of this study not previously considered in the preceding chapters.

Chapter 12: summarizes the conclusions drawn from different parts of this project.

Appendices: have been used in this instance to indicate information pertinent to this study, but which was not the original work of the author.

Due to the large number of parameters investigated and the complex nature of the possible inter-relationships between the different parameters, whenever possible a similar method of analysis to that previously presented in this study, was employed. Though this resulted in some repetition of method, the intention was to simplify the presentation and analysis of the data. In a multidisciplinary study of this sort many other parameters not central to the issue, such as diseases, external environmental parameters eg. light, nutritional composition of the feed and handling stress have been maintained constant at all treatments. Where pertinent they are discussed, though emphasis has been placed on the parameters which either the literature or experiments indicate are primarily relevant to this study.

CHAPTER 2

OBJECTIVES

The specific aims of particular parts of this study have been stated in the relevant chapters, but the general study aims can be summarized as follows:

1. To determine a method for quantifying the hydrodynamic mixing characteristics of a defined aquaculture tank.

2. To illustrate and quantify hydrodynamic mixing characteristics of a defined aquaculture tank and to investigate the effect of variations in tank maintenance, eg. water depth, flow rate, residence time and stocking density, on flow dynamics.

3. To investigate the influence of tank hydrodynamics on juvenile turbot behaviour.

4. To quantify the influence of various tank maintenance techniques, primarily alteration of water depth, on the biological indicators of fish performance and fitness, eg. specific growth rate, condition factor and food conversion ratio, and associated water quality, eg. pH, dissolved oxygen and ammonia concentrations.

5. To determine the inter-relationships between various biological, water quality and hydrodynamic parameters within a specified aquaculture tank.

6. To produce summarizing equations and a model of the interaction between different parameters acting in a tank, to indicate the relative importance of these parameters, optimal management strategies and to predict the likely effect of changes in tank management and most suitable tank designs for the culture of turbot.

Overall, this study sought to develop or indicate an improved tank design, or management strategy, for the culture of demersal turbot, rather than a pelagic species, and to study the interaction of hydrodynamic, water quality and biological parameters within a defined aquaculture system.

CHAPTER 3

REVIEW OF PREVIOUS WORK ON THE DESIGN AND PERFORMANCE OF AQUACULTURE TANKS

3.1 INTRODUCTION

Many designs of tank have been used for aquacultural purposes, some of which have been based on sound hydrodynamic, water quality, biological, economic or ergonomic principles. Others have clearly been designed without such knowledge and with no reference to the available literature. A study of that literature will not only indicate the most promising designs of tank but, more importantly, the correct management techniques and design criteria. It is important to study failed systems, because these also indicate important design and management principles, though details of these failures are unfortunately rarely published.

3.2 GENERAL INVERTEBRATE TANKS

Three designs of tank are representative of structures built to either culture or hold invertebrates for experimentation. Hale (1960), who recognised the need for water movement, suggested that the majority of nitrifying bacteria were attached to suspended particles rather than floating freely in the water. To improve nitrification he inoculated aquaria with bacteria, increased the substrate area by adding shells, rocks or

sand, maintained a species diversity for recycling purposes and ensured a water flow. An airlift was used both to move and aerate the water.

A simple invertebrate culture facility was designed by Cruz (1984) to grow large quantities of the rotifer Brachionus plicatilis. The 9000 l tanks were rectangular pyramids with a truncated bottom measuring 7.8 x 2.4 m at the top and 5.6 x 0.23 m at the bottom. A concrete frame was used as a support.

A more complex design was developed by Frutiger (1985) for the long term rearing of running water invertebrates (Figure 3.1).

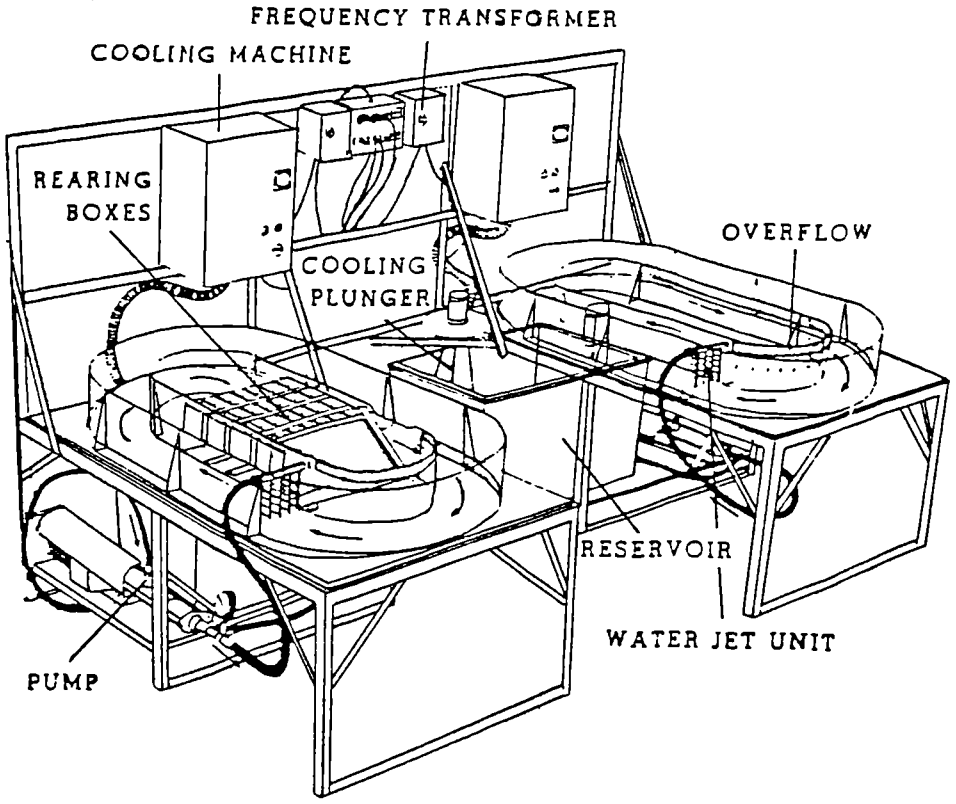


Figure 3.1: Semi-schematic representation of a flow channel. From Frutiger (1985).

Water was jetted through 16 inlets, around an arena-shaped raceway (110 x 25 x 14 cm) and out over an overflow at one end, via additional central rearing boxes to the outflow. The outflow and inflow were connected directly by a pump. The channel shape and drive produced strong turbulence but damage to drifting animals was minimal. The use of multiple inlets such as this, to distribute flow evenly, is a feature that many workers have not employed.

3.3 MOLLUSC TANKS

Like flatfish, many molluscs require space as substrate area rather than tank volume, so molluscan cultivation technology may be applicable to this study.

25.4 cm diameter polyethylene funnels with a simple airlift recirculation system have been used successfully to grow Japanese little neck clams, Tapes japonica, over a 21 day period (Langton et al., 1977). The animals were placed in a mesh tray within the funnel. Water entered the funnel tangentially above the surface and spiralled downwards past the trays at 60 l min^{-1}. No difference in growth rate was achieved by the addition of a central standpipe which caused suspended matter to settle out. This standpipe must however have caused a substantial dead volume to develop in the container if flow velocities were so slow that settlement of faeces was permitted. This decrease in the recirculating volume of water may have

been the cause of the lack of an improved growth rate despite the decrease in suspended faeces. The design also indicates that tanks with a shape which is not suitable for the culture of benthic animals may be adapted for such use.

Dupuy et al. (1977) recommended the use of stacked trays for holding oysters in a seed hatchery. Pairs of holding flumes were stacked five tiers high. Each flume contained six Nestier trays holding 5000, 6.35 - 19.05 mm (0.25 - 0.75 in) oysters. A flow of 22.73 l min^{-1} (300 gals h^{-1}) was considered the least which produced a uniform water flow across the oysters.

The culture of bivalves in flow-through raceways has been successful in tropical Pacific regions. A study of the type of tank best suited to giant clam Tridacna gigas production is currently being undertaken by the International Centre for Living Aquatic Resources Management (Govan, pers. comm.).

3.4 CRUSTACEAN TANKS

The basic requirements of crustacean culture tanks, ie. food suspension, detritus removal, uniform oxygen distribution and removal of nitrogenous waste also apply to flatfish tanks.

Mock et al. (1973) constructed raceways, for the

cultivation of Penaeus sp., with an airlift to circulate the water, but without a particulate substrate or ends in which animals could congregate. The 5.9 x 2.7 x 1.0 m raceways (Figure 3.2) with a 16 m^2, tar-coated concrete bottom, were filled with 12,000 l of water, to a depth of 75 cm.

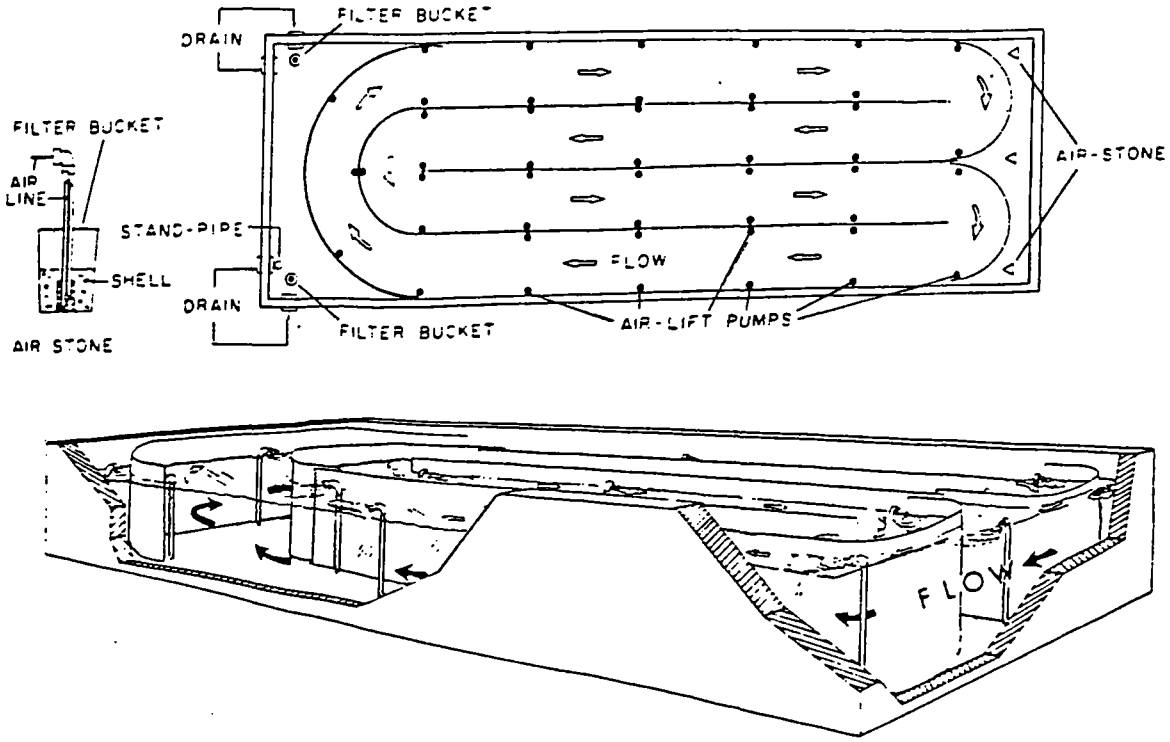


Figure 3.2: Top view and cut-away drawing of a raceway system. From Mock et al. (1973).

The water mass was divided into narrow channels by fibreglass walls. 49 airlift pumps aerated the water and produced a flow. Some of the pumps were installed on the inside of the channel curves to reduce particle deposition. 38 l min^{-1} was passed through bucket filters positioned in two corners of the tank. Water quality was considered to be good and the system held 690 g of post-

larval shrimps m^{-2} with no stratification or stagnation of the water. The system appeared to utilise regions of slow flow velocity to settle out suspended solids. The use of three internal longitudinal walls, rather than the more usual single internal wall of the sort described by Burrows and Chenoweth (1970), was probably designed to produce a more laminar flow with the available airlift pumping power.

Sandifer et al. (1974), designed a rectangular decapod larval culture tank with a sloping bottom, down which a water current was directed (Figure 3.3). An airlift at the deep end produced a counter current at the surface. A gravel filter was attached. The system maintained food particles in suspension, dispersed the larvae and was successfully used to rear Macrobrachium rosenbergii larvae.

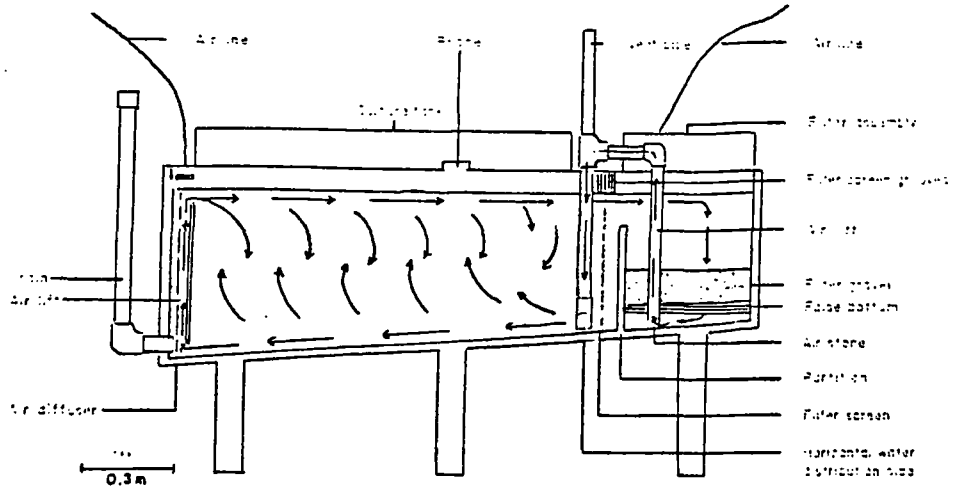


Figure 3.3: Schematic side view of rectangular culture tank with filter attached. Culture tank is 1.5 m long by 0.6 m wide by 0.5 - 0.7 m deep. From Sandifer et al. (1974).

This design was based on tank and system design criteria established by Zielinski et al. (1974). These criteria included: the removal of dead areas, formed as a result of flow separation, by forcing flows into dead spaces using baffles, pumps or making the boundary correspond to the streamline configuration; a sloping tank floor in the direction of the bottom water flow, to aid self cleaning; easy access to tanks which should therefore be capable of being grouped or stacked; the use of cheap airlifts for aeration and circulation. These recommendations highlight the need for a knowledge of tank hydrodynamics and the ability to apply that knowledge to a practical biological system.

Further work by Zielinski et al. (1976) using the same basic tank design as Sandifer et al. (1974) resulted in a more efficient Macrobrachium sp. larval tank design. Water flow was stabilized while self-cleaning and the resuspension of food was increased. An airlift at the deep end of the tank was replaced by triangular boards. Gibsonite particles were used to study the tank flow characteristics. Several conclusions were made regarding the tank design and husbandry: a single manifold inlet pipe was better than a double pipe; the inlet flow should be orientated to flow parallel with the tank flow; heavier food required a greater flow to maintain it in suspension; measured velocity, scouring velocity and the position of debris within the tank were correlated; a controlled settlement of old food could be achieved by adjusting flow

rates. A silk screen was later added to the tank to separate larvae from the waste water removal zone (Zielinski et al., 1978). 1.2 m diameter x 2.25 m deep, conical bottomed, 1800 l, cylindrical tanks for culturing M. rosenbergii larvae were also tested to determine the minimum flow required to maintain food particles in suspension. A single tangential inlet and a 30 cm diameter nylon screen outlet at the top centre of the tanks were considered optimal. Similarity laws were used to predict the performance of prototype tanks based on experimental scale test results.

Hughes et al. (1974) described an inverted bell-shaped 37 - 41 l lobster larvae culture tank. Water was introduced into the tank tangentially through slits in the base of a screened central standpipe. The rotational flow maintained the waste in suspension prior to removal via an overflow. The hydrodynamic characteristics of the tank were determined and the flow was found to approximate complete mixing. The resulting high (80%) survival of larvae was attributed to the food, larval density and hydrodynamic characteristics of the tank. This again confirmed the importance of flow dynamics in tank design.

Sandifer and Smith (1975) compared the growth and survival of M rosenbergii in 2.5 m^2, 2200 l circular tanks, with 2.4 x 1.2 x 0.6 m, 2.8 m^2 rectangular, epoxy-coated wooden raceways. After 112 days at stocking densities of 10 and 200 prawns m^2, survival was 82 and

50 % respectively in the circular tanks. In raceways stocked with 200 - 400 prawns m^{-2} survival was greater than 80 %, although growth was slow. Raceways were considered to be generally better than circular tanks for culturing Macrobrachium sp, but flow or mixing studies were not conducted.

The importance of tank design in aquaculture was highlighted by Delves-Broughton and Poupard (1976), who proposed that the prawn diseases which occurred in British institutions were caused primarily by poor tank design, poor water quality or inadequate nutrition, although this statement was not qualified.

Rectangular tanks for the culture of crustaceans have since been used with varying success. Adisukresno and Purnomo (1977) achieved only 3 - 20 % juvenile survival in 1.5 - 6.0 m^3 plastic hatchery pools. Shrimps were then transferred into 5.8 x 1.8 x 2.0 m concrete tanks under a glass roof. Macrobrachium sp. production at the Brackish Water Aquaculture Development Centre in Indonesia, which used various rectangular concrete tanks, circular plastic pools and square wooden tanks covered with plastic sheeting, was however described as, "quite impressive" (Adisukresno et al., 1980), though the relative performances of the different designs was not indicated.

Mock et al. (1973) designed and tested 5.2 x 2.4 x 0.8 m, 12.5 m^2, Penaeid sp. shrimp raceways with 24

airlifts and found them to be practical for rearing post-larvae. Two large raceways 24 x 3 x 0.9 m, 71.1 m^2, with rounded ends, a 0.5 ° slope to the tank centre and 0.3 x 0.6 m tapered rectangular drain, were also tested. At a flow rate of 15.2 l min^{-1} the water quality was often poor. A fibreglass spawning tank for Penaeid sp., 97.5 cm diameter x 90 cm deep, with a rectangular fibreglass bioassay tank, 82.5 x 50 x 50 cm, containing 50 l of water, a central airlift and a submersible pump, was later developed (Mock et al., 1980).

Two types of lobster rearing facility were described by Ingram (1985). A rearing system comprised an inflow to the system via an aerated, heated, header tank, from which water flowed by gravity into the top tray of a stack of six trays and then down through each of the lower trays. Partitioning within trays was optional. Alternatively a compact unit containing circular rearing tanks, a broodstock tank, Artemia separator and juvenile rearing trays was described.

Crustacean facilities can be divided into those for hatching and those for ongrowing. Both have different requirements. Crustacean larvae typically are pelagic and therefore need to be distributed evenly throughout the water mass, as does their food. Adults tend to be demersal so in this case the tank biomass is concentrated on or near the tank floor. It is evident that hatchery tanks have become relatively efficient compared with

earlier designs. The large scale intensive ongrowing of crustaceans however, while more efficient now than in early years, is still in need of further development. In many cases it is clear that new tank designs were not based on previous published results. This combined with little specific work qualifying tank designs has resulted in slow and repetitive development. In studies which combined hydrodynamic, biological and ergonomic principles such as those described by Zielinski et al. (1974; 1976) important developments resulted. Rectangular raceways appear the most successful design for crustacean culture, with the emphasis on increasingly larger units and greater stocking densities.

3.5 TANKS FOR ROUND FISH

3.5.1 Circular tanks

One of the first circular ponds with a central outlet was built in 1904 (Surber, 1936). Several 1.52 x 0.46 m (5 ft x 18 in) and 7.62, 15.24 and 30.48 m (25, 50 and 100 ft) diameter x 0.46 m (18 in) deep tanks were built. The 15.24 m (50 ft) diameter design was considered most practical for the culture of trout, though the reasons for this were not given.

Efficient fluid dynamics and waste removal were identified at an early stage as being important requirements for intensive culture fish tanks. Cobb and

Titcombe (1930) obtained what they considered satisfactory results using a 14 m (46 ft) diameter circular tank with a single tangential water inlet which set up clockwise spiral currents to a central outlet. Waste was removed from near the centre of the tank using a siphon. Their previous designs utilized a multi-aperture inlet pipe set across the full diameter of the tank to produce a circular flow.

Surber (1936) followed on from this work and stressed that centrifugal action only occurred in truly circular tanks and was disrupted in oval, oblong or partitioned tanks. Of the 3.66, 4.57 and 6.71 m (12, 15 and 22 ft) diameter tanks which were tested, the 4.57 m (15 ft) diameter design was preferred, though again reasons for this were not given. Various other aspects of tank design were studied. A 15 - 23 cm (6 - 9 in) gradient in the tank bottom was constructed to aid the removal of wastes. No difference in the survival or growth of trout and bass was achieved in tanks with either a gravel or concrete substrate. The preferred inlet was a 1.22 m (4 ft) long radially set pipe with flattened 2.5 cm (1 in) pipes set at 60 ^{o} to the water surface. Various central outlets as aids to self-cleaning were reviewed. One design of outlet was composed of a standpipe which could be unscrewed from its base to facilitate the release of water and waste within a standpipe screen. Southhall's-self cleaning cylinder, positioned inside the central screen, directed water through the screen, into a central well and then to

the bottom of the cylinder before removal of the standpipe. A radial gutter draining from the centre to the outside of the tank floor was also described.

Numerous designs of circular tanks have been tested, primarily with the aim of improving and producing a more even flow distribution. 2 m diameter x 35 or 60 cm deep tanks were used by the Department of Agriculture and Fisheries for Scotland for growing salmon Salmo salar alevins (Billard and Petit, 1973; Thorpe, 1981). These tanks had central water and feed inlet pipes and a screened waste trough around the outer edge of the floor. Haynes (1975) preferred to physically separate salmon fingerlings from any waste by screening off the bottom of the tank. A revolving brush, driven by compressed air, pivoted at the centre of the tank, continually scrubbed the floor beneath the screen and carried waste to a central outlet.

More recent innovations in circular tank design included the use of concentric tanks (Figure 3.4) for growing fish of different sizes (Moore, 1977). Doors between the tanks were constructed to reduce handling stress caused by transfer. Inflowing water was directed tangentially through one nozzle per tank and removed by simple floor drains. It is unclear how such a system would allow the efficient removal of solid wastes and prevent recycling of poor quality water.

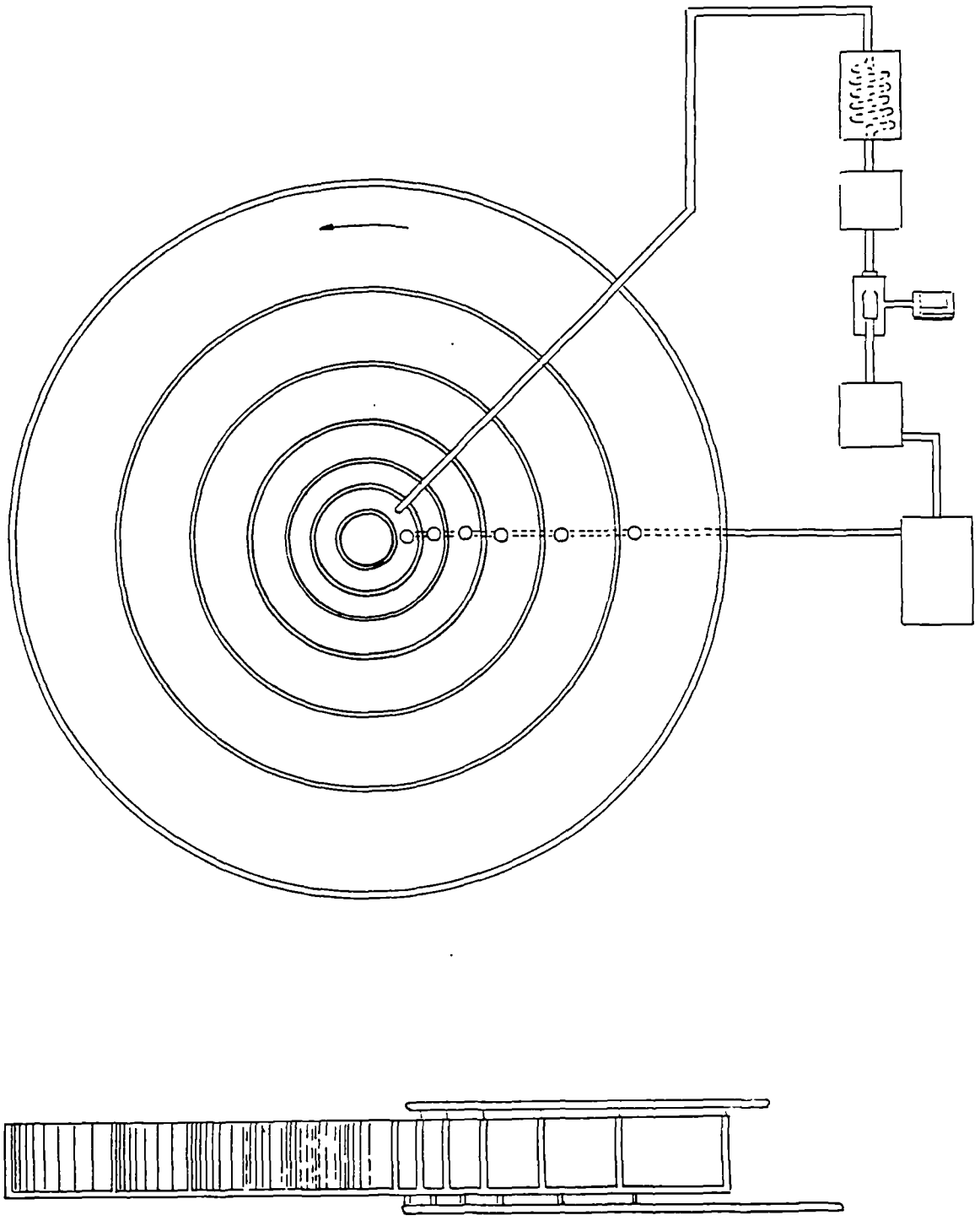


Figure 3.4: Concentric tanks for growing fish of different sizes. From Moore (1977). US Patent 4003337

A less complex tank, designed primarily to save costs, was described by Woods et al. (1981) for the culture of striped bass Morone saxatilis. The 7.3 m diameter x 1.2 m high tank with a contoured sand bottom, was constructed using polyvinyl lined galvanised steel and filled with water to the rim. The tank was easy to construct and maintain and had a long life expectancy. An inflow of 60 l min^{-1} through 6 mm jets in a PVC pipe provided extra aeration and a residence time of 10 h. The outflow was directed over a screened central standpipe. 79 % survival was achieved at a density of 13 - 52 (61-141 mm long) fish m^{-3}.

Kincaid et al. (1976) developed a small scale culture system which held fish for experimental purposes. The system was composed of three parts: an outer 92 cm diameter x 61 cm high galvanised steel tank with a central standpipe; a tank top assembly for holding individual containers in position and for distributing the inflow; and five 28 cm diameter x 35.6 cm high, 21.5 l polyethylene rearing buckets with 0.32 cm diameter holes in the bottom for drainage. The system allowed individual buckets to be treated separately.

Drawbacks of using circular tanks for round fish included the development of central dead areas and a build up of wastes resulting from sub-optimal flow rates and flow distribution, as indicated by Burrows and Chenoweth (1955) and Rosenthal et al. (1982). Many of the

disadvantages can be overcome by correct design and operation. Some of the required criteria were described by Larmoyeux et al. (1973). These included the need for correct inflow rate and direction to aid self cleaning and aeration. Jet aeration was found to be efficient. Horizontal slots rather than holes in a central screen were recommended. To economise on floor space circular tanks could be stacked or staggered in rows. Deep silo tanks, described in the following section were also suggested. This study also indicated the ways in which the hydrodynamics of tanks could influence water quality and biological aspects.

A combination of water exchange rate and photoperiod affected the growth rate of sea bream, Sparus aurata, grown in 3,000 l circular, fibreglass tanks (Tandler and Helps, 1985). Kincaid et al. (1976) showed that tank volume and available water limited the growth rate of rainbow trout Salmo gairdneri in some situations in circular tanks. They considered that such limiting factors were indicated more accurately by growth rate variations rather than growth depression, survival or dissolved oxygen levels.

Oxygen and metabolite concentrations, which are extremely important in intensive culture systems in general and circular tanks in particular, are closely associated with flow rates. Low dissolved oxygen levels can be limiting, causing a decrease in growth and survival

of striped bass (Allen, 1974). The growth of catfish in 1.58 m diameter, 1 m^3 fibreglass tanks was affected by aeration levels and water flow below a dissolved oxygen level of 3 mg l^{-1} (Carter and Allen, 1976). At oxygen concentrations greater than this figure, neither increased aeration or water flow appeared to improve growth rate or conversion efficiency.

Ammonia-nitrogen similarly reduced growth, yield and conversion efficiency in densely stocked 1.22 m^3 diameter, 460 l tanks (Brauhn et al.,1976). Levels of toxic unionized ammonia fluctuated both with position in the tank and on a daily basis (Rosenthal et al., 1982).

Both tank design and management affect water quality, particularly in circular tanks which, it has been shown, are susceptible to flow dynamics problems. They become increasingly important factors to consider as the intensity of commercial culture systems increase.

3.5.2 Silos and vertical raceways

Silos and vertical raceways are vertical tanks with a depth greater than the width. Potentially they have several advantages compared with horizontal tanks, such as reduced floor space requirements, increased flow rate, improved self-cleaning and ease of harvesting. A 2.29 m x 5.03 m (7.5 ft x 16.5 ft high) 24,821 l (5460 gal) cylindrical trout silo with a flow rate of 2,281 l min^{-1}

(500 gal min^{-1}) was found to be self-cleaning, gave a high yield, maintained greater than 5 mg l^{-1} (5 ppm) oxygen and was easily controllable (Buss et al., 1970). Production was however low in terms of the flow rate, so a non-limiting water supply was required for such a system. Similar systems, with the inflow introduced to the centre bottom of the tank through a vertical pipe, have been designed and patented (Buss, 1975). Oxygen was also added through a porous plate at the bottom of the silo and forced into solution by water pressure. The water flowed upwards to a screened overflow (Figure 3.5).

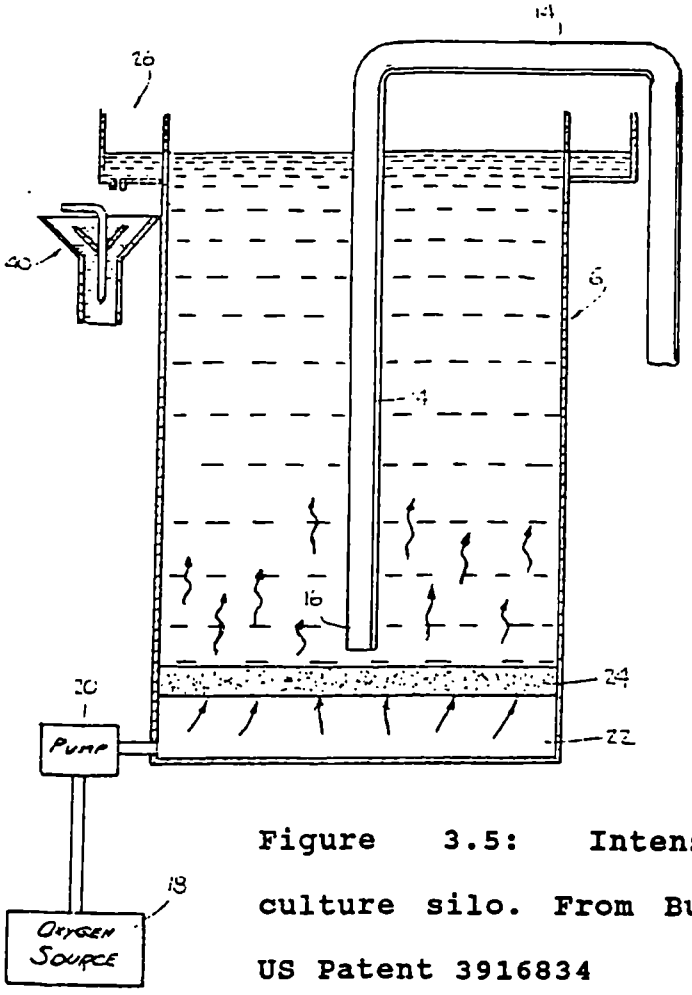


Figure 3.5: Intensive fish culture silo. From Buss (1975). US Patent 3916834

Finger (1974) suggested a similar design of silo, apparently for use in cold climates. The silo, which was housed within a building, had a reduced surface area, water and air heaters and artificial lighting. Improved ease of harvesting was claimed. The inflow was introduced at many depths through a vertical perforated pipe. The outflow was located at the bottom centre of the tank.

A more complex silo design for culturing trout or carp was presented by Fruchnicht (1975). Several removable mesh floors, sloping outwards for self-cleaning, connected to inner and outer walls, formed a multistory tank (Figure 3.6). A spiral downflow was produced by an inclined tangential sprinkler which also ensured an adequate oxygen concentration. A latticed food tray was positioned just above water level. A screened bottom drain prevented waste and fish from disrupting the outflow. Water quality and flow distribution, which would be expected to be disrupted by the shelving, were not defined. Such a design, which increased available floor area, may be useful for flatfish culture, however a high stocking density on the shelves would greatly inhibit the spiral water flow.

Later designs tended to be simpler. A cylindrical up-flowing silo, with a floor sloping to the centre and a screened overflow at the top, was found to be self-cleaning (MacVane, 1979). Waste, carried by the spiral flow, sank to the bottom and was removed through a hinged

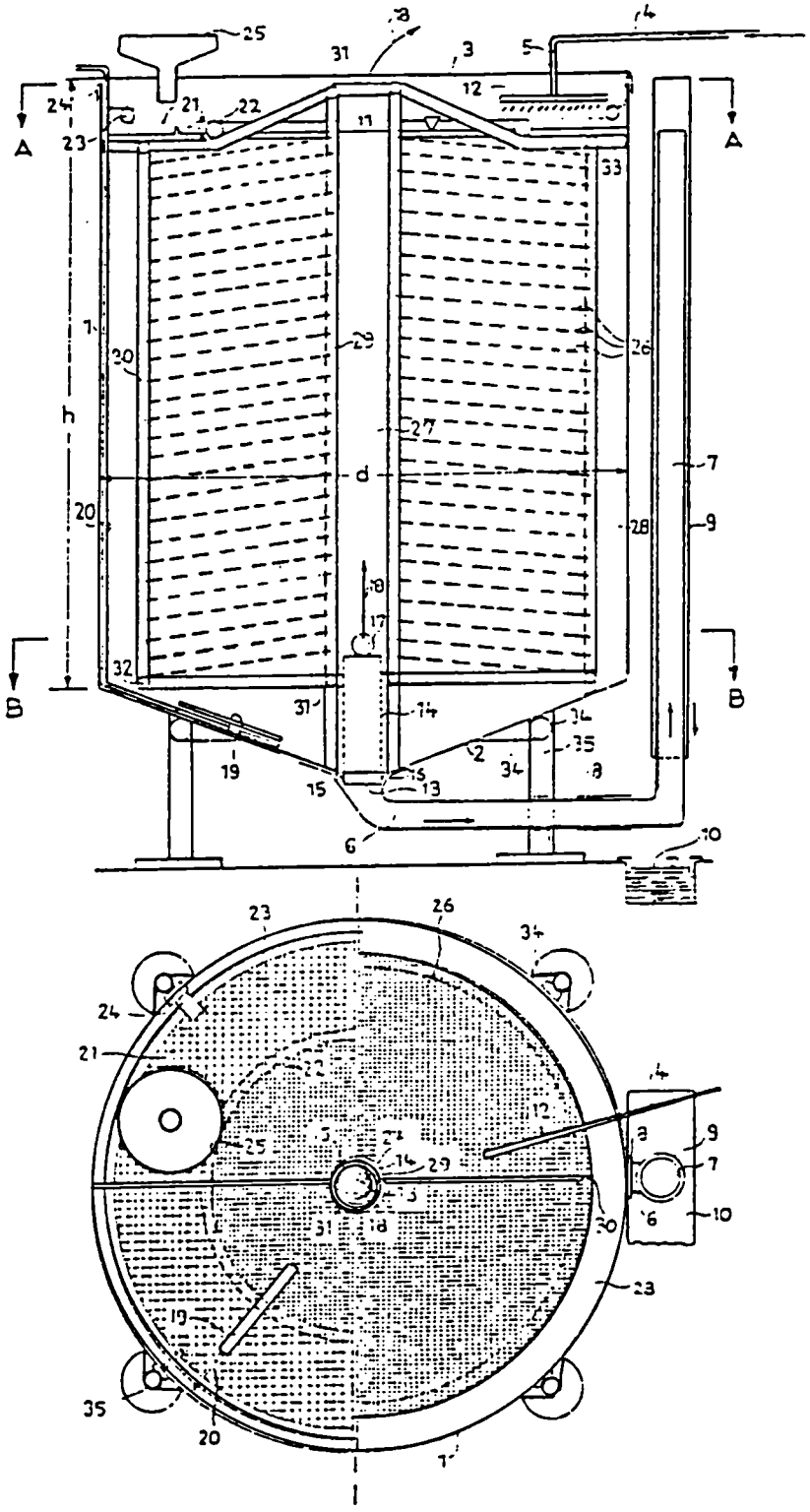


Figure 3.6: Trout or carp culture silo.
From Fruchnicht (1975).
US Patent 3870018

screened drain. A 32 l silo with a vertical perforated pipe inflow located at the side wall rather than the centre of the tank, was used to grow 595 g of channel catfish, Ictalurus punctatus, (Slone et al., 1981). A water overflow to a collecting ring was located at the top of the tank. A uniform upward spiral flow with a dead settlement area at the bottom of the silo was produced. An occasional downward spiral flow to the bottom drain flushed out solid wastes. A slow inflow rate of 2.22 l min^{-1} assisted self-cleaning. 24 - 40 l cylindrical silos with a tapered bottom, surface inflow and perforated bottle shaped outflow above the tapered area also utilised a stagnant sedimentation area at the bottom of the tank (Kuhlmann and Koops, 1981). Solid particles resulting from the eel culture settled in the waste pipe at the bottom centre of the tank and were occasionally flushed to waste. Growth rate was claimed to be improved. 450 l fibreglass conical tanks, or silos, of a similar design to that of Kuhlmann and Koops (1981), except for a 75 - 95 l min^{-1} air-lifted recirculation system, were used to grow tilapia (Leclerq and Hopkins, 1985). Daily fluctuations in oxygen, pH and ammonia were distinguished.

Large scale tower tank systems have been constructed to study planktonic ecosystems (Balch et al., 1978). A 3.05 m diameter x 10.09 m, 73 m^3, plastic coated Scripps tank, with 15 cm diameter viewing ports, was lined with insulating polyurethane foam. The Dalhousie tower tank (Balch et al., 1978), constructed of PVC-lined reinforced

concrete was 3.66 m diameter x 10.46 m high, with viewing ports, six inlet levels and five outlet levels. The tank was acoustically uncoupled on neoprene pads to prevent vibration.

3.5.3 Raceways and rectangular tanks

Raceways have been used and developed over many years for the culture of a wide range of aquatic organisms, but mainly for growing fish such as trout and catfish. It is expected that some properties of raceways, eg. flow characteristics and a large floor area, may be particularly relevant to the culture of flatfish and indeed have been used for this purpose by Golden Sea Produce Ltd., at Hunterston, Scotland.

Water circulation, aeration and screen size were three criteria, affecting the efficiency of rectangular tank designs, studied by Haskell *et al*. (1960). "Hot spots", areas of low oxygen level which fish avoided, were found to develop in tanks with an uneven circulation. It was suggested that to avoid such design faults engineers and biologists should work together when developing tanks.

Buss (1981) confirmed the need to study the effects of tank design on water quality. He suggested that tanks should be designed around the amount of oxygen available. The limiting factors of metabolites, pH and temperature were considered almost as important as water flow. It was

also proposed that the stocking density of a tank was an important factor to consider and that exchange rates and stocking density of a raceway, divided into separate units, will be decreased, resulting in increased wasted space, construction and maintenance costs. As an example it was suggested that salmonid densities could be increased from 48 to 128 kg m^{-3} if the criteria stated above were optimised. Further research into the fundamental environmental requirements of fish, minimum tolerable oxygen levels and the effects of environmental parameters on conversion efficiency was suggested.

From an engineering viewpoint, water circulation and in particular the requirement of smooth flow dynamic characteristics, to produce rapid mixing of influent and tank water, are important criteria in tank design (Westers and Pratt, 1977). A gradient in water quality was considered desirable, and this implies that dead areas may be retained.

Tank hygiene has long been considered an important design development criteria. Rodgers (1949) tested various raceway flows to improve self-cleaning and to give a choice of water velocity. 6.71 x 1.52 x 0.15 m (22 x 5 ft x 6 in) tanks, with a raised bottom and gradient to a mid-line 30 cm (1 ft) deep trough, were developed and required no cleaning other than algae removal during a 23 day trial. Three wood baffles set across the tank increased water flow near the floor. The installation of

baffles in a tank for increasing bottom flow (Westers and Pratt, 1977), producing an even flow distribution (Burrows and Chenoweth, 1970; Christensen and Chenoweth, 1974), or directing particles to waste (Schlieder, 1984) can contribute considerably to self-cleaning. Other rectangular tanks have been specifically designed to be self-cleaning (Westers, 1979; Fordham and Gelin, 1986), or for ease of cleaning (Brett et al., 1971; Anon., 1978; Charlon and Bergot, 1984). Alternatively the maintenance of a sufficiently high water exchange rate can be used to assist self-cleaning (Murai, 1979; Buss, 1981).

The uneven distribution of fish within tanks is a problem associated with raceways. Tanks have been designed which give an even flow (Haskell et al., 1960; Burrows and Chenoweth, 1970; Christensen and Chenoweth, 1974) and so distribute fish evenly, or present a gradient in water quality allowing fish a choice of position (Westers and Pratt, 1977). With criteria such as those suggested by Haskell et al. (1960), Westers and Pratt (1977) and Buss (1981) a considerable development of raceway design has been achieved.

Haskell et al. (1960) studied aspects of the design of three types of pond used in New York State hatcheries. "Bath" ponds were constructed in two sizes, 15.2 x 4.6 x 0.6 m, 35.4 m^3 (50 x 15 x 2 ft, 1,250 ft^3), with a 7.6 cm (3 in) slope from end to end, and 30.5 x 9.1 x 0.91 m, 200.9 m^3 (100 x 30 x 3 ft, 7,100 ft^3). The inlet and

outlet ends of both sizes of tanks were tapered. Fluorescein dye flow tests indicated that water circulation was poor in both sizes of tank, but particularly in the large tanks. Water short-circuited from the inlet to the outlet leaving dead, non-mixed areas to one side of the inlet. The dimensions of "Rome" type ponds were the same as the Bath ponds, but with no tapered ends and with three spillways across the entire width of the ponds, at both the inlet and outlet. The flow characteristics of the Rome ponds were superior to those of the Bath ponds. Dye injected into the inflow was distributed throughout the tank. "Dirt" type ponds, 18.3 x 3.7 x 4.6 m (60 x 12 x 15 ft), with sloping sides, a disc-shaped cross section and a 0.91 m (3 ft) wide inlet and outlet, had a poorer circulation than either Bath or Rome types. It was concluded that spillways at the inlet and outlet of the tanks should be symmetrical and free of obstructions to give an even flow distribution.

A pair of 2.4 x 0.6 x 1.2 m (8 x 2 x 4 ft) plywood raceways, with biological and mechanical filters, and a recirculation rate of 30 - 35 exchanges h^{-1}, ie. 75.7 l min^{-1} (1,200 gal h^{-1}) was adequate to maintain an intensive fish culture (Reed et al., 1973), but details of the flow dynamics and its effect on growth rate were not stated. The need for such a high flow rate implies that either the tank was not efficiently designed or operated.

80 x 30 x 15 cm troughs filled to a depth of only

5 cm were used to culture 30 - 706 mg channel catfish fry (Murai, 1979). By maintaining a high flow rate of 12-18 l min^{-1} and a high density of up to 467 g l^{-1}, suspended waste particles were kept in suspension until flushed from the trough. Despite intermittent periods of low oxygen concentration a 2200 % weight gain was achieved with a greater than 97 % survival rate during a 6 week period. The decrease in water depth with the aim of decreasing the residence time in a tank, to increase flushing will be examined in the present study.

Rectangular, fibreglass 4.3 x 3.0 x 1.2 m, 13,000 l tanks, with rounded corners and airlifts at each corner were used to culture gag Mycteroperca microlepis (Schlieder, 1984). Debris was collected at the bottom of a triangular settling tank and baffles were used to remove large particles. A rectangular tank shape was considered most efficient on floor space. Water circulation was not restricted by this shape and the brood stock acclimatized well.

Behaviour (Honer et al, 1987b), growth (Honer et al, 1987c) and both long and short term fluctuations in water quality (Honer et al, 1987a) in 42 l rectangular tanks for growing juvenile tilapia Sarotherodon galilaeus were studied. Increased water flow improved water quality to some extent, while increased stocking density decreased water quality. The tanks were similar in size to those used in the present study.

Other novel designs of rectangular fish tanks include: a tank containing two, 3 l, partially screened compartments which could be removed for cleaning and for transferring fish larvae (Charlon and Bergot, 1984); a round-ended 182 l (40 gal) tank with a multi-jet inlet and central standpipe outlet for use in energy budget studies (Brett et al., 1971); and a PVC coated cloth pond suspended between galvanized pipes. The dimensions of the latter design were 3 - 10 x 1.5 x 0.96 m, with a water depth of 75 cm. Other rectangular tank systems are described by Wheaton (1972), Petit (1981) and Brown (1983).

The production of the rectangular circulating raceway, or Foster-Lucas pond, of the sort described by Burrows and Chenoweth (1955), was a significant advance in the development of rectangular raceways, despite initial problems. It was stated that design influenced carrying capacity, oxygen and CO_2 concentrations, metabolic waste levels, disease inhibition, food distribution and self-cleaning. An investigation into the hydrodynamic, biological and physical parameters associated with this type of tank revealed that the design caused poor water circulation, as measured by visualization studies (Burrows and Chenoweth, 1955). Turbulence occurred at each D-end, resulting in low velocity areas and short circuiting. Such problems with water circulation can then be regarded as significant. A previous study by Palmer et al. (1952) did not however indicate reduced growth rates in this type

of tank, compared with other tanks. Moreover the uniform distribution of fish and velocity of water, which were obtained to improve fish stamina, have been considered advantages of the Foster-Lucas pond (Burrows and Combes, 1968).

An improvement to this design was developed and tested by Burrows and Chenoweth (1970). Their 15.2 - 22.9 x 2.4 x 0.76 m (50 - 75 x 8 ft x 30 in) rectangular circulating pond comprised, as did the Foster-Lucas pond, a central dividing wall, around which water flowed (Figure 3.7).

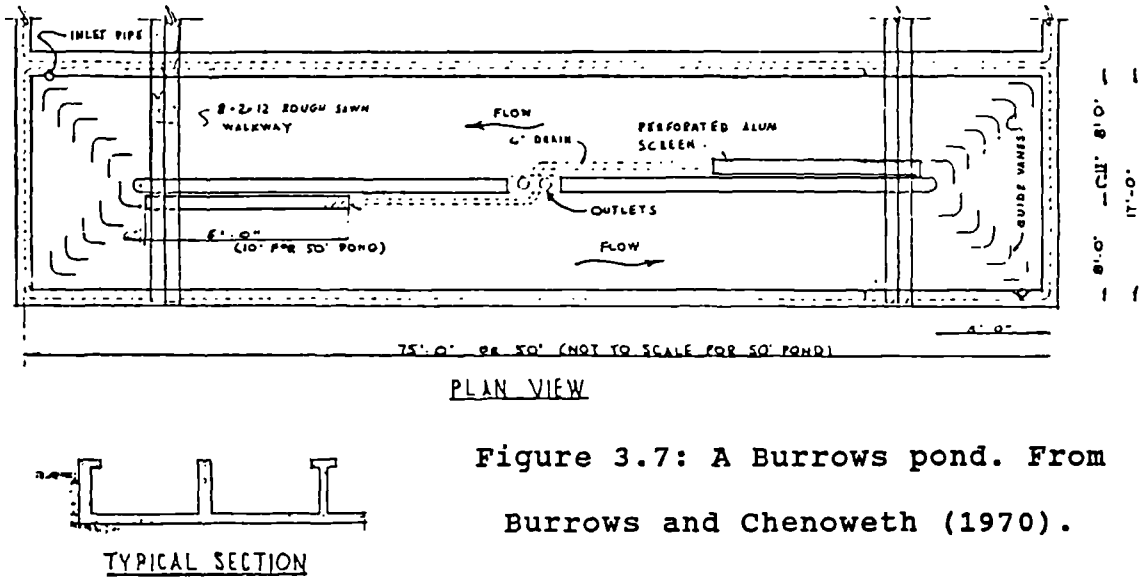


Figure 3.7: A Burrows pond. From Burrows and Chenoweth (1970).

Guide vanes at each corner and two, 15 cm (6 in) diameter drains covered by perforated aluminium screens leading to a central outlet, were added. The vanes reduced the turbulence caused by water turning at the ends of the raceway. The drains were located in the suspended particle settlement areas caused by water turning around

the ends of the centre wall. Two sets of jet inlets were located on the outside walls of the tank. The tank was self-cleaning and improved the survival and quality of salmonids cultured in it.

Similar designs, known either as Burrows or Foster-Lucas ponds, have since been used (Christensen and Chenoweth, 1974; Westers and Pratt, 1977; Fordham and Gelin, 1986).

3.6 OTHER NON-FLATFISH TANKS

Numerous designs of tanks for holding or culturing eggs, larvae, fry or adults of fish have been described. An early review of salmon and trout hatchery design (Nordqvist, 1893) described various facilities for holding fish eggs including Williamson wooden egg troughs which were stacked in horizontal or vertical rows, through which water was forced up from below. Stacked systems have the advantage of saving floor space. 1.2 - 1.8 m (4 - 6 ft) diameter, circular tanks fastened by a swivel to a vertical post and stacked five high (Figure 3.8) have been used to grow 1000 - 2000 trout fingerlings per tank (Prevost, 1941). Such a system appears suitable for flatfish culture because the tank floor surface area, within a given volume or floor surface area of fishfarm, has been increased by a factor equivalent to the number of stacked tanks.

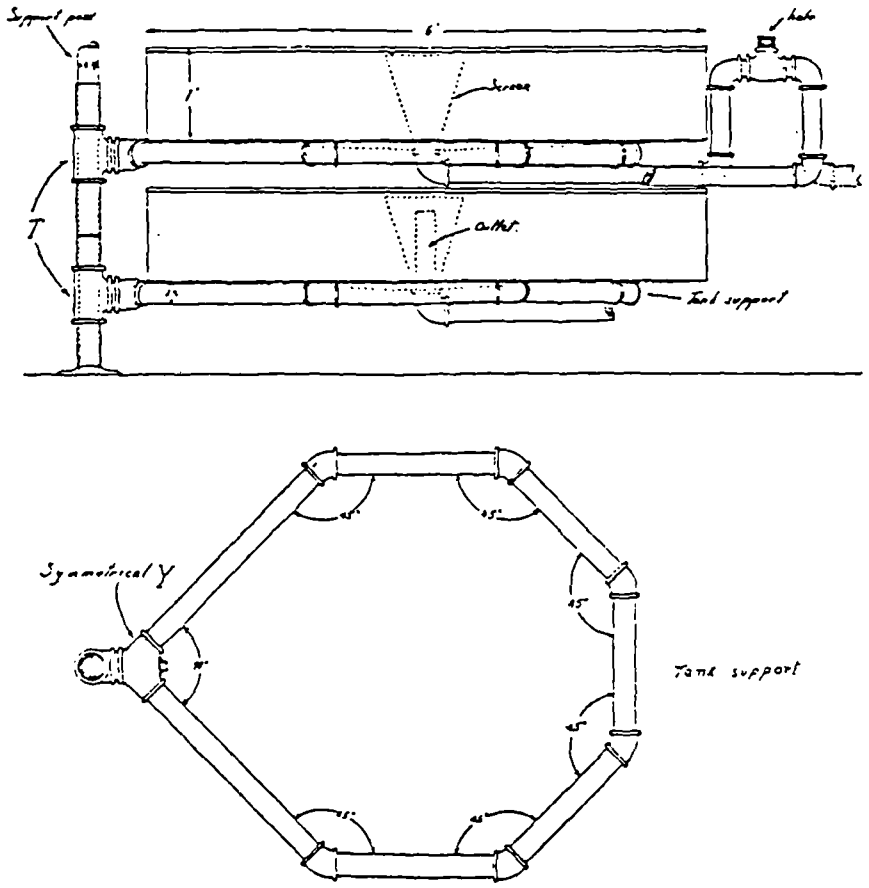


Figure 3.8: Details of supporting cradle and swivel attachment for superimposed circular hatchery tanks. From Prevost (1941).

A flexible low cost system for maintaining salmonids, composed of three columns and comprising a reservoir above four vertically stacked 6 l containers was proposed by Larrivee et al. (1983). The unit enclosure was constructed of fibreglass coated plywood. A 0.2 l min^{-1} flow rate was required to minimize temperature and oxygen fluctuations.

A dual freshwater fish tank and tomato growing system was proposed as a means of utilizing fish metabolites (Pierce et al., 1981). The plants were located above a 24 x 1.2 x 0.6 m rectangular tank. Such a system would not

be possible in seawater because of the low tolerance of higher plants to salinity, though algae may be used in marine applications (Tollervey, 1984).

Adapted 46 cm (18 in high), navy surplus drop tank nose cones were suitable for holding trout swim-up fry (Gahimer et al., 1971). Water flowed from a tangential inflow above water level to a central standpipe outflow. The tank was partially self cleaning and held 1 kg of fish in 27.3 l (6 gal) of water for 6 months. Small scale container designs used for experimental purposes, such as those described by Wolf and Piper (1960) are not relevant to this study.

3.7 COMPARISON BETWEEN NON-FLATFISH TANK TYPES

While the development of various tank designs continues, opinion has varied as to which tank was most suitable for various different applications. Comparisons between circular tanks and rectangular tanks, or raceways, were frequently made. Palmer et al. (1952) compared the growth of salmon in circular and Foster-Lucas ponds and found no difference. The growth of salmon was however greater in 4.9 x 4.9 x 3.0 m (16 x 16 x 10 ft) troughs than in 1.8 m (6 ft) diameter circular tanks (Johnson and Gastineau, 1952). Circular tanks have been preferred for some uses because of their self-cleaning properties in restricted flow conditions and lower mortalities compared with 2 m^2, round cornered, central draining, square tanks

(Pyefinch, 1970). A more even water flow and fish distribution, and therefore better self-cleaning, was also attributed to circular tanks, compared with some raceways (Burrows and Combes, 1968; Brown and Gratzek, 1980). Conversely a comparison between the biological and physical aspects of circular tanks, Foster-Lucas ponds and raceways revealed that the performance of circular tanks was bad, Foster-Lucas was poor and raceways were the best (Burrows and Chenoweth, 1955). At that stage of development however circular tanks or Foster-Lucas flow dynamics may not have been optimised.

An important point to note is that square and rectangular tanks are more efficient spatially in a fishfarm than circular tanks. Klapsis and Burley (1984) calculated that the usable surface area of round cornered square tanks was 21 % greater than circular tanks.

Many authors have grown fish in more than one type of container, ie. circular tanks and raceways (Allen, 1974), square tanks and raceways (Lemercier, 1981), circular tanks, rectangular circulating ponds, square tanks and raceways (Westers and Pratt, 1977; Piper et al, 1982), circular and rectangular tanks (Leitritz and Lewis, 1976) and a wide variety of tanks (Balarin and Haller, 1983; Kafubu and Ikeoue, 1983), but have not quantified comparisons in terms of physical and biological parameters.

It is difficult to determine from the literature, which is often contradictory, the type of tank most suitable for a particular purpose. This conclusion arises from the constant developments in tank design and the various different methods employed to determine tank efficiency. Some of the designs reviewed above may be applicable either directly, or with some alteration, for the culture of flatfish. Silos and other tanks without a flat floor and with a great depth of water will be inefficient spatially for turbot, though modifications may improve their chances of use. Circular tanks, used extensively at present for many aspects of fish farming, have intrinsic problems as a result of dead areas and short circuiting, but may continue to be useful with careful management, because of their low cost. Rectangular raceways tend to use more water than circular tanks but have simpler flow dynamics and fewer associated problems. The Burrows pond is little used at present, but may prove effective for growing flatfish, particularly in recycle systems, because of the even flow distribution and efficient use of water momentum, flow dynamics and usable surface area.

3.8 FLATFISH TANKS

A lack of information currently exists regarding the design of tanks for culturing, or even just holding, flatfish. What information there is, primarily concerns

egg or larval culture, such as that described by Shelbourne (1963), Shelbourne et al. (1963) and Howell (1972). In the majority of studies, eggs were hatched and larvae reared in rectangular tanks.

Plaice were grown in 1.5 x 0.6 x 0.6 m tanks filled to a depth of 43 cm at a flow rate of 27 l h^{-1}. The survival rate through metamorphosis was approximately 3 %. Improved water quality increased the number of plaice surviving metamorphosis (Riley and Thacker, 1963). 3.65 x 3.65 x 1.22 m fibreglass tanks have been used to spawn sole, while plaice were spawned in large shallow ponds (Shelbourne, 1970; 1976). Plaice have been grown at densities of 25 kg m^{-3} in onshore tanks compared with 73 kg m^{-3} in sea cages, though practical densities were probably 40 - 80 % of this (Kerr, 1974).

900 l tanks with a flow rate of 10 - 40 l h^{-1} at 15 ^{o}C were used to study the effects of photoperiod on the growth of flatfish (Alayse and Lahaye, 1979; Alayse, 1981).

Most of the tanks described above were designed to hold the pelagic larval stages of flatfish. It is clear from the small amount of work carried out on tank design for the demersal stages of flatfish, that there is considerable scope for development in this field.

3.9 TURBOT CULTURE

The majority of the literature regarding turbot culture has been concerned with economic feasibility and water quality (section 1.2). Details of specific tank developments for turbot, or as stated previously, the demersal stages of flatfish, have not been found. Where details of tank designs have been mentioned, they have been included as background to other experiments.

During an early turbot cultivation trial, Purdom et al. (1972) held 223 wild caught fish in twelve, 30 l tanks and 169 fish in a 200 l tank, with flow rates of 30 and 300 l day^{-1}. At 7 months the fish were graded into 5 x 3 x 1 m pens which were part of a 65 m^3 recirculation system. Using this system at 18 ^{o}C a market size of 30 cm, from 5 cm initial length in 14 months, was achieved. The fish were found to be easy to manage and did not develop hierarchies even in the large tanks. This is an important factor to consider when designing turbot tanks.

Trials by the White Fish Authority (WFA) (1975a) at Hunterston utilized 1.8 x 1.8 x 1 m cages with plasticised mesh sides and a solid fibreglass bottom within rectangular butyl-rubber lined 40.6 x 3.7 x 1.0 m timber tanks. Stocking density was 136 fish m^{-2}. Fish were later released into penned sub-divisions of 18 m^2 at 47 fish m^{-2}. 13.8 kg of fish m^{-3} were produced in 54 m^3

tanks with a stocking density of 12 fish m^{-3}.

Further work by the WFA (WFA, 1977; Smith, 1979; WFA, 1979) at the Ardtoe recirculating nursery unit was carried out using 1000 l, 2 x 2 x 0.3 m asbestos tanks. The resulting conversion rates were 1.14:1 - 1.71:1 (wet weights) at 96.5 - 100 % survival and a maximum stocking density of 20.25 kg m^{-3}, but twice daily cleaning was required. These tanks and the water treatment system can therefore be described as reasonably successful. However Thain (1979) in a summary of work carried out by the WFA between 1976 and 1978 stated that rectangular butyl rubber lined tanks caused irregular water movements and therefore promoted unhygienic conditions. 2.5 m diameter, 4.5 m^2 tanks containing 1.35 m^3 of water and a flow rate of 1.9 m^3 h^{-1} were described. Wild caught fish fed in mid-water, whilst hatchery fish fed on the surface.

Spectorova and Doroshev (1976), who reared black sea turbot S. maeoticus maeoticus, suggested that conditions within 60 l trays may have been less stable than in 2 m^3 tanks. This conclusion may confirm work carried out by the WFA, detailed above, which indicated successful culture conditions in large tanks.

Poxton et al. (1982) achieved conversion efficiencies of 3:1 wet feed: live fish weight increase and a 2 % day^{-1} growth rate during ongrowing in rectangular 0.37 m^2 tanks filled to a depth of 20 cm. Poxton et al. (1981) also

described a 1110 l tank with a sloping floor for carrying waste to an integrated filter. Water was recycled using airlifts.

Turbot larval rearing tanks have been described by Howell (1973), Jones et al. (1974) and Olesen and Minck (1983). Various other researchers have cultured turbot to quantify growth (Jones 1974; Person-Le Ruyet et al., 1980), survival (Purdom, 1973) or profitability (Anthony, 1908) but have not described the holding facilities used. Parameters which affect growth or survival of turbot are important tank design and testing criteria. These parameters have been reviewed in sections 8.5.8 - 8.5.13.

The WFA (1975b) concluded that important improvements were required, in flow rate, feed, ongrowing density and the labour required, for the tank culture of sole and turbot. This review indicates that while many of these factors have been individually improved, research into flatfish tank design has been neglected. Such work is particularly important, as indicated by the extensive literature regarding other species. Many factors such as water quality, fish fitness and performance, stocking density and labour, are influenced by tank design and management, so the potential implications and benefits of this work are wide ranging.

An estimation of the possible designs of tank which may be best suited to turbot culture, based on the small

amount of available turbot specific literature, cannot be made. Other than the suggestion by Thain (1979) that the rectangular tanks used by the WFA may be unsuitable, no firm conclusions can be drawn, hence the need for further work. This review does however indicate the principles involved with tank design and management, the factors which must be considered and the possible designs, adaptions and management strategies available.

3.10 UNSPECIFIED AND MISCELLANEOUS AQUACULTURE TANKS

This section reviews literature relating to non-aquaculture holding tanks, tanks for which the aquaculture use has not been specified, and general comparisons between several types of tanks.

Weatherley (1983) described some of the basic design principles of aquaculture tanks. He stated that aquaculture was traditionally a low technology industry, but that market demands for fish were justifying intensification. This leads to a reduction in space requirements, lower water demands, independence from natural cycles, including weather, and regulation of optimum conditions. Recirculation systems give extra control to the producer, while flow-through systems require a plentiful supply of good quality water. A superior tank design, with good mixing and solids removal, has minimal stagnant regions and an even distribution of oxygen, metabolites and food, although this is difficult

to achieve. He further suggested that flow visualization can be used to study dynamic characteristics, and mathematical modelling will improve design.

Other system design criteria were described by Saeki (1958). He calculated that within a tank, a weight of water 2 - 11 times the fish weight was required to alleviate adverse water quality conditions. Kerr (1981) lists six factors which determine tank size and type: maximum stocking density, initial density, fish size range, growth rate, growth variation and frequency of grading. Equations for calculating the output and water requirements of a farm were listed and a partial review of aquaculture equipment was included. Kinne (1976a) also reviewed equipment and details of water treatment.

Rectangular tanks not reviewed in previous sections include those used for incubating fish eggs. Floor space was considered by Burrows and Palmer (1955) to be an important factor influencing hatchery construction costs. Vertical incubators have been described by Burrows and Palmer (1955) and Senn et al. (1973).

Varadi (1984) presented various calculations relating physical and water quality parameters to the growth of fish in raceways and other flow-through systems. It was proposed that as actual fish stocking density increased with growth, the water volume in a tank should be kept within a range which avoids stress but maintains self-

cleaning. It was also suggested that water depth should be altered, to maintain a constant water velocity throughout the growing period.

Stickney (1979), describing circular tanks in general, considered that this design, as opposed to raceways, did not have dead spaces. This theory disagrees with extensive work carried out by several authors including Burrows and Chenoweth (1955), Osbourne (1981) and Rosenthal et al. (1982), who described a central dead space in circular tanks. A venturi drain system with two concentric standpipes was considered the most useful method of self-cleaning which worked best in tangential flows. Two other suggestions relevant to turbot tank design were that rectangular tanks utilised floor space more efficiently than circular tanks, agreeing with the calculations of Klapsis and Burley (1984) stated earlier, and that large tanks were cheaper in terms of floor area than small tanks. The problems associated with such tanks include improper mixing, short-circuiting, irregular flow patterns resulting in oxygen depletion and ammonia accumulation and insufficient self-cleaning of particulates. It was thought that many tanks had been designed for simplicity rather than as a result of understanding.

Of the numerous aquaria described, three are most relevant. To avoid upstream turbulence in a constant cross-sectional flow channel Vogel and LaBarbera (1978)

used a propeller rather than a water pump. A trough length to width ratio of between 10:1 and 5:1 gave a smooth water flow. Tanks with a ratio in excess of 10:1 produced a non-uniform boundary layer. Hale (1964) also described a fast flow aquarium while Jackman (1974) gave a comprehensive account of the construction and maintenance of marine aquaria.

3.11 Ancillary equipment

Ancillary equipment pertinent to tank designs include water inlets and outlets. Other equipment, important in the running of an aquaculture system, eg. mechanical pumps, filters etc. are not usually housed within a tank and so have not been reviewed. Within tank structures have been mentioned previously, when the design and purpose of the tank in which they were housed was described. This section reviews these structures specifically and does not repeat previous discussions.

Two types of tank inlet water regulators appear relevant to this study. Kinghorn (1982) stated that a cistern based design, controlled water more accurately than the use of a flow meter. The position of an adjustable overflow maintained a constant head of pressure. Both coarse and fine adjustments in water flow were possible. A multi-purpose jet flow regulator described by Oron *et al*. (1983) adequately controlled

dissolved oxygen and ammonia levels in a high density tilapia culture.

An early improvement in outlet drain design was the replacement of a standard internal standpipe overflow system with a bottom draining standpipe surrounded by a protective screen sleeve designed by Surber (1933). This system removed settled particles from the tank floor. Burley and Klapsis (1985) incorporated a venturi bell-mouthed internal standpipe within the fish screen. This alleviated turbulent effects and produced a steady exit vortex motion.

Parker (1980) preferred an external standpipe arguing that internal standpipes interfered with dip nets and accumulated solids on the bottom of the tank. Advantages of the external system included: quick adjustment of water level, rapid flushing and emptying, effective particle removal and a lack of valves.

Surber (1936) studied the effects of the position of a standpipe within tanks. It was suggested that side outlets were not as good as central outlets because the former may disturb the self-cleaning action of a circular tank. A trough in the tank floor from the centre to the side outlet was tested but was found to trap fish and waste.

Though often overlooked, screens surrounding internal

tank drains are an important aspect of tank design because they can influence water flow and waste particle distribution. Stephens and Evans (1950) designed a 76 cm (30 in) high, 15 cm (6 in) diameter metal cylinder screen fitted with a crank and shaft so that the size of the opening at the base could be regulated. This however quickly rusted so it was replaced by a square perforated corrosion-proof screen. Self-cleaning action was lost but all fish were retained and the screen did not become clogged with debris. Surber (1936) also preferred a central square screen outlet which stopped waste moving within the outlet recess. External and internal standpipe screens with horizontal and vertical slits were tested by Osbourne (1981). An 8 x 6 mm vertical slit screen around a central standpipe gave the most uniform water velocity profile. Burley and Klapsis (1985) found that wedge shaped vertical slits in the central screen improved flow in round-cornered 1 m^2 square tanks.

3.12 CONCLUSIONS

Often aquaculture tanks are designed by biologists without a full appreciation of engineering principles, or by engineers with little knowledge of the requirements of the cultured species. Burrows and Chenoweth (1955; 1970) are examples of how these two fields of study should be combined. Burrows and Chenoweth (1955) first evaluated the suitability of three types of rearing pond based on the hydrodynamic characteristics. Factors influencing

model size and flow patterns in models and prototypes were examined, using flow visualization and dye concentration studies. These were then related to the physical and biological characteristics, such as carrying capacity, disease inhibition, food distribution and cleaning efficiency.

Many tanks for pelagic fish have been proposed. Of these, few have been specifically tested for their effectiveness at culturing a species. In many instances located, the flatfish tanks used have been merely pelagic fish tanks with little or no adaption. It is expected that the environmental requirements of demersal flatfish will be markedly different to those of round fish and other groups. No work can be found which relates tank design to biological parameters of flatfish. Tanks, an obviously vital part of an aquaculture system, are often taken for granted. The effects of tank design on growth rate, stocking density, disease control, self-cleaning, survival, product quality and water quality are often overlooked, or deleterious effects attributed to other factors. A detailed study into hydrodynamic, water quality and biological aspects of tank design and their relation to flatfish in general and turbot in particular is therefore urgently required.

Silos and vertical raceways have a low floor area to volume ratio, unless adapted as described by Fruchnicht (1975), while rectangular raceways without substantial

adaptions were found by Haskell et al. (1960) to be prone to uneven flows and fish distribution. Usually, large flow rates were required to alleviate this problem (Murai, 1979). In general, the most suitable designs of fish tank currently available appears to be circular with a central outlet, because of its more even flow distribution (Burrows and Combes, 1968; Brown and Gratzek, 1980) and better self-cleaning in restricted flow conditions (Pyefinch, 1970) compared with other designs. It has been shown that the deleterious flow dynamic effects can be alleviated by careful management, as demonstrated by Larmoyeux et al. (1973). Though more wasteful in terms of fish farm floor surface areas, some form of stacking, also suggested by Larmoyeux et al. (1973), may be possible, particularly if the weight of water in a tank can be reduced for a demersal species. The work that has been conducted with turbot (Jones et al., 1974; WFA, 1977; Smith, 1979; WFA, 1979) indicates that greater success was achieved with larger tanks, greater than 1000 l, possibly because small tanks have less stable conditions (Spectorova and Doroshev, 1976). If currently available fish tanks are to be used to culture turbot, large carefully managed circular tanks appear most suitable.

It should be noted that the Burrows pond, a rectangular circulating raceway with several innovative adaptions to aid flow distribution, self-cleaning and water conservation (Burrows and Chenoweth, 1970) looks particularly suitable for turbot cultivation, but its use

in Europe appears limited, possibly because of its more complex design.

Though costly or complex, the most suitable design of tank for a particular species, or prevailing conditions, may give substantial benefits which include: improved species survival, fitness and performance; reduced pharmaceutical additives; reduced pumping costs or conservation of water; reduced feed wastage; and reduced labour costs. Over the lifetime of a tank, the benefits may far outweigh the increased cost of the tank. The progressive fish farmer or researcher should not therefore base the choice of tanks merely on initial capital cost, or the appearance of simplicity because, as this review has indicated, the tank is a vital and often under-appreciated piece of aquaculture technology.

CHAPTER 4

GENERAL MATERIALS AND METHODS

Methods and equipment which have been used on several occasions during this study will be described in this chapter. Materials and methods specific to one area of the research have been detailed in the chapter to which they refer.

4.1 RECIRCULATION SYSTEM DESIGN

4.1.1 Overall system design

Various aquaculture systems, in which juvenile turbot may be grown, have been described (Kinne, 1976b; White Fish Authority, 1979; Poxton et al., 1982). Aspects of water quality and stocking densities required and resulting turbot growth rates or food conversion efficiencies achieved in given systems have also been studied (WFA, 1975a; Jones et al., 1980; Poxton and Allouse, 1982; Poxton et al., 1982; Kleinot, 1986). These workers and others have shown that recirculation and flow-through system design has developed sufficiently to produce high nursery and ongrowing growth rates (see also section 8.4.5).

The primary function of the integral units of flow-through or recirculating systems, eg. filters, temperature regulators, pumps etc., is to provide optimum, or at least

maintain adequate, water quality conditions, required for survival and growth. Considerable research has been carried out into the various aspects of these integral units, including that carried out by Howard and Kingwell (1975), Kinne (1976a), Jones et al. (1980), Weatherley (1982) and Weatherley (1984).

Recirculation systems, in which a proportion of the effluent water is recycled back into the system, are particularly useful for aquaculture research because water quality and environmental conditions can be controlled more accurately than in systems which are constantly reliant on an external source of water. Recirculation systems are however more technically difficult to operate, because aspects of water quality have to be carefully monitored and adjusted. Of particular importance are DO and unionized ammonia concentrations.

To undertake the experiments detailed in this study, a closed recirculating mariculture system was designed and constructed. Several design criteria were applied to the unit, in addition to those normally required to culture fish. Twelve identical fish treatment tanks each with independently adjustable flow rates and water depths were needed. A large sump volume to buffer changes in system water quality was advantageous. The system had to fit into the available space on site and contain as much of the equipment already obtainable at the facility as possible. An adaptable design for reuse in several

experiments was required. Cheapness was an important consideration.

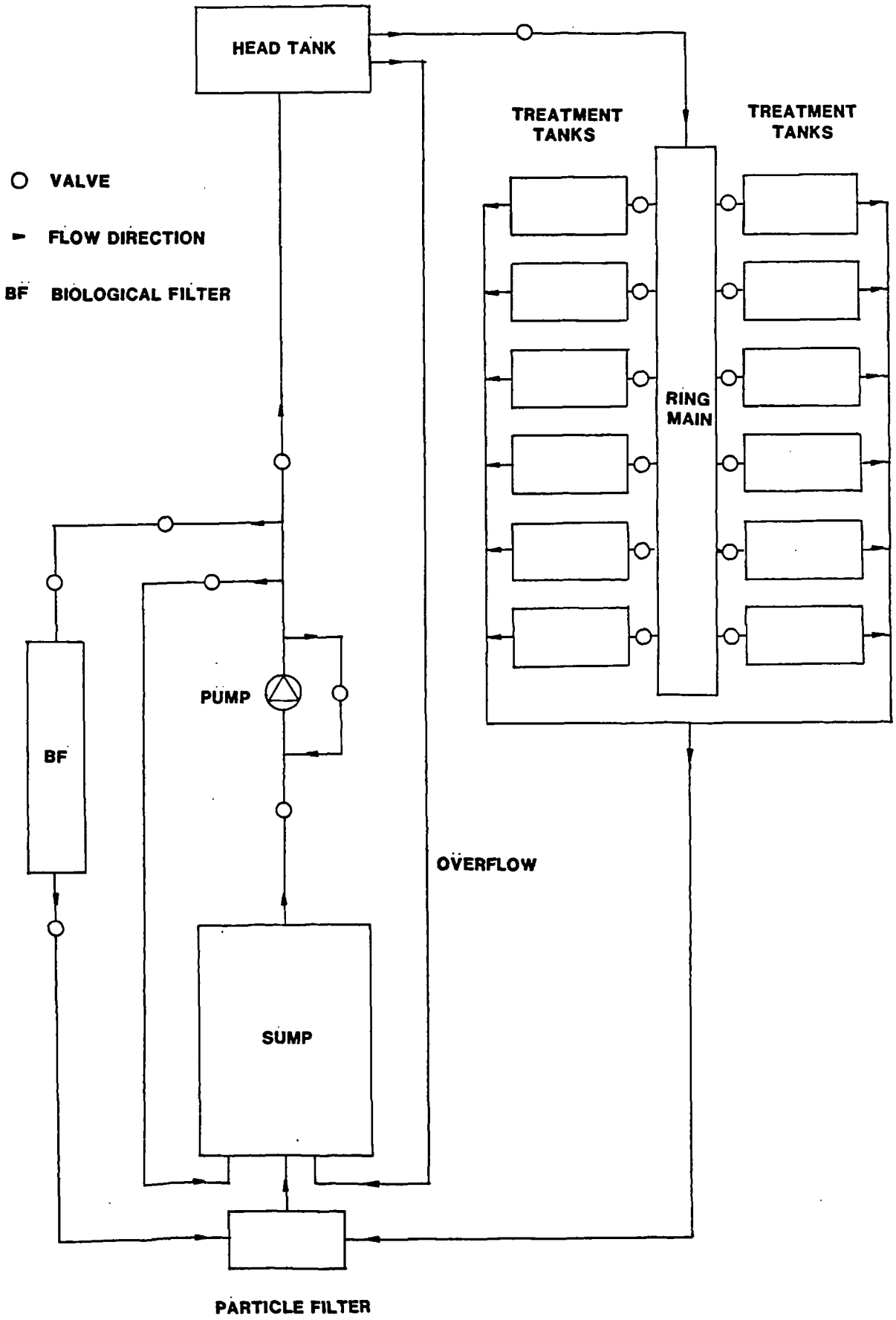
To economise on space and maximize the use of available equipment, the unit was constructed around a 3500 l, fixed, rectangular sump tank which had previously been used to ongrow turbot. Stock treatment tanks were located on a scaffolding platform, constructed above the sump tank using "Kwikstage" system scaffolding (C. Evans and Sons Ltd., Newbridge Industrial Estate, Newbridge, Edinburgh).

A simplified flow diagram of the experimental recirculation system shown in Figure 4.1, indicates the general design used, with only minor variations to suit different experiments (Figures 5.4 and 5.5). Water was pumped from the sump around three main loops.

Incorporated into the water treatment loop was a cylindrical biological filter (Table 4.1), the base of which was 1400 mm above ground level and which received pumped water directly. 0.167 m^3 of the 0.186 m^3 filter (90 %) was filled with limestone chippings. Water re-entering the sump from the filter and stock loops was passed through a 0.04 m^3 activated carbon and sand filter.

Water passed around the stock loop via a 0.239 m^3 constant head tank which supplied a ring main and equalized water pressure to twelve treatment tanks

Figure 4.1: Simplified flow diagram of recirculation culture system (not to scale)



containing the turbot. Effluent water from the treatment tanks was collected in two open 63 mm (2.5 in) diameter uPVC drain pipes and passed through the sand and carbon filter to remove particulate matter, before returning to the sump.

A continual flow of water through a head tank overflow pipe was maintained to achieve a constant head of water to the stock ring main, so that steady flow rates into the treatment tanks were ensured and the risk of flooding was minimized. The 51 mm (2 in) diameter overflow pipe passed directly back to the sump tank, with the minimum impedance to flow.

Outflows from, and inflows to the sump were located at opposite ends, so circulation within the sump was improved. Aeration was applied to the head and sump tanks rather than directly to the stock treatment tanks, because the air stream produced turbulence in the water which disturbed flow. A consequent risk of low DO levels in the treatment tanks during water pump failure was accepted. Aeration in the sump tank was located so that air bubbles were not carried into the pump inlet which would increase the risk of supersaturation. Aeration of the head tank water reduced the possibility of supersaturation.

A Vanton centrifugal water pump was used in the system (Figure 4.1). A voltage regulator ensured a constant pumping output, to a maximum of about 28 l min^{-1},

at the head of 4480 mm required in this system. Output was not however stable, but in practice, the maintenance of a constant flow through the head tank overflow pipe ensured that a constant water pressure at the treatment tank inlet valves was maintained.

All closed pipework and fittings were grey uPVC pressure fittings. 13 mm (0.5 in) internal diameter pipework was used for individual treatment tank influents and effluents. 25 mm (1 in) internal diameter pipework was used on the treatment loop, between the sump and head tank, via the water pump and for the treatment tank ring main. 51 mm (2 in) internal diameter pipework was used for the overflow from the head tank and the ring main supply from the head tank. Pipes and fittings were bonded with either Tangit PVC cement, UBM brand PTFE thread seal tape, or General Electric contractor's silicon sealant, which contained no fungicides. Wherever possible, pipework was sealed with silicon sealer or PTFE tape so that the fittings could be reused. Fittings bonded with PVC cement or silicon sealer were not pressure tested until 24 h after bonding, so leakage of water through the seal and leaching of solvent into the system water was reduced.

Upon completion of the recycle system, freshwater was pumped around the system for 5 days and then discarded, to ensure that solvents and particles of uPVC were removed. Table 4.1 summarizes the dimensions of the system units.

Table 4.1: Recirculation system hydraulic data

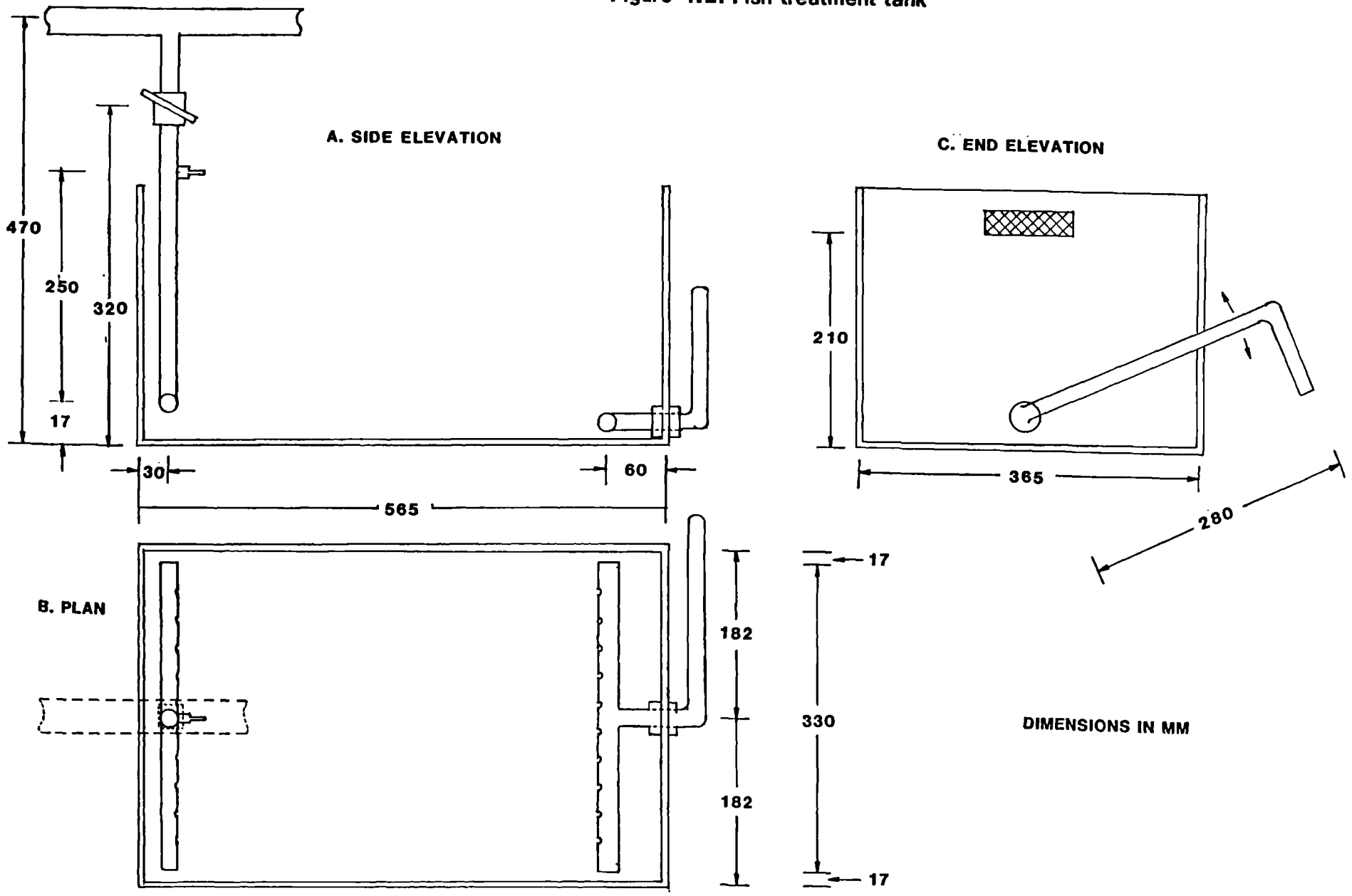
unit	no.	length (m)	width/ diameter (m)	depth (m)	max working volume (l)
sump	1	3.040	1.200	1.200	3500
biofilter	1	-	0.400	1.480	186
head tank	1	0.900	0.600	0.600	239
mechanical filter	1	0.565	0.365	0.235	40
treatment tanks	12	0.565	0.365	0.235	37
estimated total volume excluding pipes					4409

4.1.2 Fish tank design

Twelve grey, high density polyethylene (HDPE), 50 l stacking containers (Stakrak, 121 Lynchford Road, Farnborough, Hants) were used as fish treatment tanks throughout this study (Figure 4.2). The containers were not designed as fish tanks and so could not be considered particularly suitable for commercial culture purposes, primarily due to their small size and rectangular shape. The containers did however have several advantages for use in this study: the rectangular shape allowed maximum use of space on the scaffolding platform; the HDPE material was smooth and easy to clean; they were inexpensive; the dimensions of all 12 treatment tanks were effectively identical; and it was considered useful to show that with careful adaption and management, even poor tank designs could be successfully utilized for fish culture.

The hand-hold holes in the end walls of the tanks were covered with netting (Figure 4.2c) to prevent the

Figure 4.2: Fish treatment tank



loss of fish during accidental flooding. The netting allowed water to overflow in extreme circumstances, but retained the fish.

A 330 mm long, 13 mm (0.5 in) diameter inlet spray pipe, across the upstream end of the tank, was constructed to improve flow dynamics within the rectangular tanks and therefore optimize the tank volume available. The pipe, which was stoppered at the ends, was perforated with ten 4 mm diameter inlet holes spaced 30 mm apart. The holes faced horizontally towards the outlet end of the tank. An identical collection pipe was located at the downstream end of the tank, with outlet holes facing horizontally towards the upstream end of the tank. To reduce the quantity of water passing directly from the inlet to the outlet (short circuiting) along the bottom of the tank, the inlet pipe was located higher above the tank floor than the outlet pipe.

The supply of water into each tank, from the communal ring main, was independently adjustable, through 13 mm (0.5 in) ball valves (Figure 4.2a). The height of water in the tanks was adjusted by rotation of an external standpipe about a screwed tank connector joint connected through the tank wall to the collection pipe. To reduce splashing of effluent water whilst passing from the standpipe to the communal effluent collection drainpipe, a flexible down-pipe was fitted to the standpipe (Figure

4.2c). A hole was drilled into the highest part of the standpipe assembly to prevent siphoning.

4.2 GENERAL HUSBANDRY

Exact husbandry routines varied slightly between experiments. Small differences in routine, and methods specific to an experiment, are described in the appropriate chapters. Many of the husbandry techniques were however employed throughout this study.

4.2.1 Feeding and cleaning

The general method employed to feed the fish is described in section 4.4.2. The order of the tanks in which the fish were fed was varied daily. Normally feeding occurred once per day at 12.00 h.

Whole pellets were selected from the petri-dish food container appropriate to a particular tank. Broken pellets, not of the standard pellet size, were not fed to the fish. The pellet was broken in half and the two halves were dropped into the tank. During feeding the fish were examined for signs of disease, abnormality or death. Care was taken to ensure that all fish had the opportunity to feed. When the fish did not continue to take the feed, feeding was discontinued in that tank. Pellets assigned to fish in one tank were not offered to

fish in another, so that the quantity of food given to each tank was known.

Tanks were cleaned, normally 1 h after feeding. The period between feeding and cleaning was a compromise between allowing the fish to digest the food undisturbed and the necessity of tank hygiene. The order in which the tanks were cleaned was varied. Uneaten pellets, faeces and any other debris were removed using a siphon tube with a soft flexible tubing tip to avoid damage to the turbot. Care was taken to avoid disturbing the fish greatly, but most of the turbot were moved during cleaning to ensure that no debris was concealed by the bodies of the fish. The number of pellets or half pellets removed by the siphon from each tank was noted. Water containing the debris was passed through filter matting to filter out the particulate matter and then returned to the sump. This method ensured that no system water was lost, irrespective of the time taken to clean the tanks.

While the siphon was efficient at removing particulate matter, a surface film built up on the walls and floor of the tank during the days between weighing. Removal of fish for weighing was used as an opportunity to scrub the tanks, while the fish were absent, to remove this film.

4.2.2 Water quality adjustments

Recirculation system seawater was maintained at approximately 35 ppt by the addition of measured quantities of dechlorinated freshwater three times per week, after the measurement of salinity during the morning.

If necessary, pH was adjusted at the same time as salinity. Measured quantities of sodium hydrogen carbonate were mixed into solution with dechlorinated water which was added to correct salinity. A pH of approximately 7.8 was preferred as a compromise between the local ambient seawater pH of approximately 8.1 and the need to lower pH values to reduce the proportion of unionized ammonia present.

Gross adjustments of temperature were achieved by alteration of the fish farm air temperature. Day length was maintained at ambient periods.

4.3 FISH STOCKS

To reduce the further stress soon after transportation, fish were not weighed on transfer from the hatchery, so the exact initial size distribution of fish was not known. Initial stock weights therefore refer to the beginning of the experiment in which the fish were grown.

Stock 1 was obtained from Golden Sea Produce of Hunterston, Scotland. The 152 post-weaned turbot were in the size range 2.3 - 10.9 g. The fish were transported in dark plastic bags partially filled with hatchery seawater and with air displaced by pure oxygen. The bags were packed in bins containing ice and transported by van for approximately 2 h. Stock 2 was also obtained from Golden Sea Produce of Hunterston, Scotland. The 130 juvenile turbot were in the size range 4.1 - 30.9 g. The same method of transport as that described for stock 1 was used.

The same method was employed to transfer each fish stock from the transport packages to the recirculation system. The packages were handled with the minimum of physical damage. Under low light conditions the fish, in sealed plastic bags, were floated on the surface of the water in one of the treatment tanks. After a 20 min period of temperature equalization the bag was perforated. Water inside the bag was gradually mixed with recirculation system water and then the fish were gently tipped out of the bag into the treatment tank. It was ensured that no fish were trapped in folds of the bag. The tank was partially covered to reduce light levels and fish were left overnight with the minimum of disturbance. This method of transport and transfer were considered adequate because the deaths of only 3 fish could be attributed directly to these causes. The mixing of water from another facility was not desirable, but the gradual

method of transfer described was considered preferable to handling or netting. Had acute levels of disease been present in incoming fish it was expected that this would have been transferred by the fish themselves, irrespective of the quantity of water transferred.

4.4 FEED

4.4.1 Preparation

Feed was prepared in 2.5 or 3 kg batches, to ensure that storage time before use was minimized. A semi-moist pellet of standard size, 1.5 cm long x 4 mm diameter was manufactured.

The apparatus consisted of a Hobart (model MFG) mincer/processor, uPVC pelletizer disc and a ten blade pellet cutter. The following ingredients were used: fresh whiting (*Merlangius merlangus*), Ewos herring (*Clupea harengus*) meal, 30 g of Peter Hand mineral mix (87.6 % ash, 20 % salt, 10% magnesium, 13.14 % calcium and 8.00 % sodium), carrageenan sodium salt, cod liver oil. Table 4.2 indicates the quantities of each constituent used in the diet.

2 kg of gutted and washed whiting was homogenised by extrusion through a steel disc with numerous 2 mm diameter holes, using the Hobart processor. Excess whiting was required at this stage to allow for retention by the

mincer. The dry constituents, carrageenate, fish meal and mineral mix, were blended in the mixing bowl of the processor until homogeneous. Cod liver oil was added and the mixture was stirred further. 1.5 kg of the gutted whiting was stirred into the mix. The completed diet was passed through the mincer to ensure homogeneity and to reduce particle size.

Table 4.2: Pellet menu

constituent	weight (g)	proportion of total (%)
minced whiting	1500	50.0
fish meal	1345	44.8
carrageenate	65	2.2
cod liver oil	60	2.0
mineral mix	30	1.0
	3000	100.0

Using a method devised by Lake (pers. comm.) this mixture was finally extruded through a 4 mm diameter pelletizer disc, on to a moving conveyor belt, producing seven approximately 1 m long parallel strips of feed during each extrusion. These strips were cut into pellets of a specific length using a purpose made pellet cutter. The cutter consisted of 10 parallel razor blades embedded 1.5 cm apart in a block of wood. To prevent the pellets drying out and becoming brittle, due to the heat resulting from the extrusion process, a maximum of 80 ml of water was sprayed onto the pellets before freezing. Pellets were manufactured with surplus carrageenate binder so that their form was retained during storage and tank cleaning,

for conversion efficiency estimates. The cut pellets were chilled on a flat tray and then stored frozen at -18 ^{o}C in sealed plastic bags prior to use.

Percentage moisture, calculated for each batch of food prepared, ranged between 37.8 - 42.1 %. Proximate analysis of the feed was outside the scope of this study. Calcedo Juanes (1988) analysed a feed, termed "whiting control", similar to that described in this study (Table 4.3).

Table 4.3: Proximate composition of a similar feed to pellets used in this study
(Calcedo Juanes, 1988)

	wet weight %	dry matter %
moisture	37.71	-
dry matter	-	62.29
crude protein (N x 6.25)	42.08	67.56
lipids	7.75	12.45
carbohydrate *	3.39	5.43
ash	9.07	14.56
NPN	-	-
gross energy (K cal g^{-1})	3.18	5.10

* = carbohydrate calculated by difference
NPN = non-protein nitrogen

Acceptance problems were observed, associated with the smallest 2.5 g turbot which were unable to swallow the full 1.5 cm long pellets. To rectify this problem, all pellets offered were broken in half, by hand, to produce an approximately 7.5 mm long pellet. Both halves of the full size pellet were introduced to the same tank, so

approximate division was adequate for conversion efficiency calculations. The design of pellet cutter used in this study was not suitable for producing pellets of a length less than approximately 10 mm.

4.4.2 Feeding method and food consumption calculations

Approximately 1 h prior to feeding, pellets were removed from frozen storage and weighed to the nearest 0.01 g into the number of petri-dishes corresponding to the number of treatment tanks occupied by fish. Each petri-dish was labelled with the number of the tank to which the food would be offered. At the beginning of an experiment the weight of food required was estimated, knowing the biomass of fish and expected food conversion efficiency and growth rate. A factor of 50 % was added to the estimated requirements to allow for error and wastage, so ensuring that the fish were fed to satiation. As the experiment progressed the weight of food required was estimated with increased accuracy, based on previous requirements, but it was ensured that a surplus of food remained after feeding, to maintain satiation. The petri-dishes were covered, to prevent evaporation, and the pellets were allowed to thaw.

Fish in the tanks were fed in a random order each day. After feeding, the weight of food remaining in each petri-dish was measured. The weight of food offered to the fish was the difference between the weight of food in

the petri-dish before and after feeding.

During cleaning, the number of pellets siphoned from each tank was noted. All pellets were of a similar size, so the individual weight of each pellet was known. The weight of pellets not eaten by the fish was therefore the product of the pellet weight and the number of pellets removed. Subtraction of the weight of uneaten pellets from the weight of food offered, resulted in an estimate of the weight of food consumed.

This method of estimating food consumption was expected to be less accurate than methods which involved the removal, drying and subsequent weighing of uneaten food, but had advantages. The method used in this study was easy to use, was not inaccurate as shown by the consistent pattern of daily food consumption in each tank and was unlikely to include faeces in the weight of food removed from the tank. A problem associated with this method was the production of pellets of the same size. Though equipment was made which reduced the cutting effort, this part of the process was time consuming.

4.5 BIOLOGICAL ASPECTS

4.5.1 Fish weight and length measurements

The period between weighing the fish during the experiment was a compromise between the requirement for

data showing changes in biological parameters and the need to reduce the stress due to weighing, so that stress due to other aspects such as water depth was not masked. A 14 day period between measurements was adopted.

To reduce the stress of weighing, and measuring fish length, a method was developed in which the time fish were retained in water, during the process, was maximised. The equipment required consisted of a Sartorius 1216 MP top pan balance, a 1 l glass crystallising dish, 1 l beaker, two 15 l plastic aquaria, a small knotless fish net, a plastic tray, paper towels and a waterproof measuring board. No anaesthetic was used or was necessary.

The tanks containing fish were sampled in a random order. Fish were netted out of a treatment tank and placed in a partially filled plastic aquarium for transfer to the weighing equipment. The crystallising dish was filled to a depth of approximately 2 cm with recirculation system water poured from the 1 l beaker. The outside of the dish was dried, prior to weighing without fish present. The dish was placed next to the tray and a netted fish from the plastic aquarium was allowed to slide from the net into the dish. In this way the fish was not handled and transferred only surface moisture into the weighing dish. Drying of the fish was not conducted because this method was expected to produce only negligible benefits in terms of increased accuracy of weighing and was expected to stress the fish. The net was

held over a tray which retained wetness away from the weighing dish, during transfer of the fish from the net to the weighing dish. The outside of the dish was dried and then dish, water and fish were weighed. Fish weight was calculated as the difference between the weight of the dish + water and the weight of the dish + water + fish.

The dish containing the fish in water was then placed on the measuring board and orientated so that the length of the fish could be measured through the transparent bottom of the dish. The fish was required to remain still during this process. Minimising water depth in the dish if the fish did not become passive, usually caused inactivity. After weighing and measuring, the fish was placed in a second aquarium partially filled with system water. When all fish in a tank were weighed and measured they were returned to the appropriate treatment tank, the walls and floor of which had been scrubbed in their absence. Fish were kept out of their treatment tank for as short a period as possible, normally less than 30 min.

4.5.2 Condition factor (CF)

In a review of CFs, Bolger and Connolly (1989) indicated that four forms of index have been used to study the CF of individual fish. Two indices are mainly used. Fulton (1911) suggested the use of the following CF:

$$K = \frac{W}{L^3}$$

where: K = condition factor

W = weight of fish (g)

L = length of fish (mm)

The use of this index was described by Bolger and Connolly (1989) as simple and appropriate, indicating that it is easy to interpret and consistent with its general meaning. The equation assumes isometric growth, ie. growth with unchanged body proportions and specific gravity. Poxton and Goldsworthy (1986) have however indicated some allometric growth in turbot during a five month period. The other main CF used was suggested by Huxley (1924) who used:

$$w = al^n$$

where: n = the exponent

a = a constant

Beverton and Holt (1957) used the former method of calculating CF because the latter method was influenced by small variations in the data. The period of the experiment was short with respect to the life of the fish and comparisons between the CFs of fish in different treatments were in most cases made during the same period. Differences in CF between fish as a result of allometric growth were therefore likely to be small. The measure of

fish fitness under different treatments using the equation of Fulton (1911) in Bolger and Connolly (1989) was therefore considered the most suitable, simple estimate of CF and so was adopted in this study.

K was multiplied by a factor of 10^5 so that CF values approximated unity. Throughout this study, the CF of individual fish was calculated, though particular fish were not identified. Individual fish condition factor changes between weighing periods could not therefore be calculated. Group (eg. pigmentation type or tank) mean CF values, calculated as the mean of individual CFs, not from mean weights and lengths, were determined to indicate CF changes.

4.5.3 Fish growth

Biomass in each tank was calculated as the sum of the individual fish weights. Biomass change was the difference in biomass between two weighing periods. Division by the number of fish in each tank resulted in the mean individual fish biomass change.

Specific growth rate (G_W), which indicated the incremental change in body weight per unit time, expressed as a percentage day^{-1}, was however used to quantify changes in fish weight. G_W as described by Jobling (1983) was calculated using the equation:

$$G_W = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \times 100$$

where: W_1 = turbot weight at the beginning of the period

W_2 = turbot weight at the end of the period

t_1 = time at the beginning of the period

t_2 = time at the end of the period

This measure of growth has been widely used by many authors including those detailed in section 8.5.4. An inherent feature of the calculation is that specific growth rate decreases with increased size, as described by Brody (1945) and von Bertalanffy (1957). Jobling (1983) described an alternative method of calculating the growth of fish which allowed for this inherent decrease in growth potential. The method is particularly useful for comparing different populations which have a large weight difference. The size ranges of fish stocks compared during the present study were relatively narrow, compared with the maximum size range possible and the maximum period of growth studied was 10 weeks. It was therefore considered reasonable to use specific growth rate as a quantification of fish growth.

Due to problems identifying individual fish, the weight change of particular fish during a period was not known. G_W values were therefore calculated from mean weight change data. The mean G_W values which were calculated referred to groups of fish of the same pigmentation type within a tank and to all fish within a

tank. Mean G_w values for a greater number of fish than this, such as all fish of the same flow rate or residence time were calculated only for summarizing purposes. The growth periods of approximately 14 days were adopted, as described in section 4.5.1.

4.5.4 Coefficient of variance

Coefficient of variance (Vu) as described by Purdom (1974) is the ratio between the variance and the square of the mean as calculated using the equation:

$$Vu = \frac{sd^2}{u^2}$$

where: sd = standard deviation

u = mean

Vu was calculated to quantify the extent of variation in the distribution of fish weights within a treatment tank.

4.5.5 Food conversion ratio

Food conversion ratio (FCR) is the ratio between fish weight gained and food consumed. Expression of the resulting ratio varies. Many authors, including Smith (1979), have described food conversion in a ratio format of weight of food consumed : wet weight increase (eg. 2.8 : 1) whilst other authors, such as Heap and Thorpe (1987) have shortened the expression to a description of

the weight of food consumed to produce a unit increase in fish wet weight (eg. 2.8). The latter method was adopted in the present study and calculated as described by the equation:

$$FCR = \frac{\hat{E}F_{t_1-t_2}}{W_{t_2}-W_{t_1}}$$

where: $\hat{E}F$ = total weight of food consumed (dry or wet weight g)

W_t = fish live weight (g)

t1 = time at beginning of period (days)

t_2 = time at end of period (days)

Comparison of FCR values quoted in this study, with other workers and their applicability as measures of fish stress, is described in section 8.5.6. Food consumption values, the calculation of which is described in section 4.4.2, and therefore FCR values, were determined on a tank mean basis, not for individual fish. A limited analysis of FCR data was carried out because of this limitation on the data, combined with the weaknesses associated with the use of ratios in statistical analyses.

4.6 WATER QUALITY ASPECTS

4.6.1 Temperature

Temperature of the system water was measured using an alcohol filled thermometer. This type of thermometer was

considered safer for the fish than a mercury thermometer, had a leakage of thermometer fluid occurred. The temperature of easily accessible regions of the recirculation system was measured with the thermometer immersed in situ. In more inaccessible regions, such as the sump, water was removed for measurement in a 1 l beaker previously immersed in the system water to equilibrate it with the water temperature. Temperatures were noted to the nearest 0.2 ^{o}C because it was considered that this interval could be distinguished by eye. Several temperature measurement regimes were conducted and these are described in the relevant chapters. For husbandry purposes the temperature of water in the recirculation system sump was measured daily at 11.30 h.

4.6.2 pH

Two methods of measuring pH of the recycle system water were employed. The majority of measurements involved the removal of approximately 100 ml of system water from the region of the recycle system under study. The pH of this water was measured using a Pye Unicam pH meter. Prior to measurement, the probe was calibrated with proprietary pH 7 and 9 buffer solutions. The pH of water removed from the recirculation system was measured within 30 min of removal. In situ measurements of pH were also made, using the same pH meter and probe as described above. Continuous measurements were obtained by the attachment of a chart recorder to the pH meter. The probe

was calibrated using pH 7 and 9 buffers before and after the period (eg. 24 h) of measurement.

4.6.3 Salinity

Water salinity was measured using a Parr Digital Density Meter DM46, which determined the specific gravity of the fluid. Salinity was expressed as parts per thousand (ppt). The salinity of water, extracted from the recirculation system, was measured within 30 min of removal to ensure that evaporation losses did not influence the result.

4.6.4 Dissolved oxygen (DO)

Due to the rapid change in DO levels in water removed from culture systems, this parameter was always measured in situ using a Yellow Springs Instruments Model 57 dissolved oxygen meter, calibrated in saturated air prior to use. Meter readings automatically compensated for variations in salinity, which was set on the meter prior to use. Measurements of oxygen concentration in mg l^{-1} from the meter were converted into percentage saturation using the equation:

$$\% \text{ saturation} = \frac{\text{Conc} \times 0.7}{\text{satn}} \times 100$$

where: Conc = measured DO concentration (mg l^{-1})

0.7 = conversion factor from mg l^{-1} to ml l^{-1}

satn = solubility of oxygen in seawater at a particular temperature and salinity (ml l^{-1})

4.6.5 Total ammonia

Care was taken to reduce contamination of samples used in ammonia analysis. Gloves were worn during the sampling procedure, all containers and equipment which were in contact with the samples or reagents were acid washed for 24 h and analytical grade reagents were used throughout. Details of the sampling procedures during each experiment are described in the chapters to which the samples refer (ie. sections 8.3.6 and 9.3.6).

The analytical method used to determine total ammonia concentration was described by Parsons et al. (1984). Details, a critique and the accuracy of the method are presented in Appendix 1. Their "alternative method" is described as: seawater is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside which acts as a catalyzer. The blue indophenol colour formed with ammonia is measured spectrophotometrically. Total ammonia concentration was measured as mg NH_4-N l^{-1}.

4.6.6 Unionized ammonia

The fraction of total ammonia which is in the form of unionized ammonia (NH_3) is dependant on pH, temperature,

salinity and total dissolved solids. Unionized ammonia concentrations were calculated using tables produced by Skarheim (1973). Unionized ammonia concentration was measured as $\mu g\ NH_3\text{-}N\ l^{-1}$.

4.6.7 Nitrite

The method described by Parsons *et al*. (1984) was used to determine the concentration of nitrite present in recycle system water samples. The outline of the method is summarized as: the nitrite in seawater is allowed to react with sulphanilamide in an acid solution. The resulting diazo-compound is reacted with N-(1-naphthyl)-ethylenediamine and forms a highly coloured azo dye. Details, a critique and the accuracy of the method are presented in Appendix 2.

This method was less prone to contamination and was more robust than the method used to determine total ammonia. Details of the sampling procedures during each experiment are described in the chapters to which the samples refer (ie. 8.3.6 and 9.3.6).

4.7 STATISTICAL ANALYSES

An IBM PC compatible spreadsheet, VP Planner (Anon, 1986) was used for data manipulation, simple statistical

analyses and trial graphical presentation. A VAXcluster mainframe facility, using primarily a Minitab package (Ryan et al., 1985), was used for more advanced statistical analyses. A full account of the theory and derivation of the statistical analyses used in this study is beyond the scope of this thesis, however a brief explanation and references are detailed.

The acceptance level of statistical significance throughout this study was described as a probability level of $p<0.05$. If a greater level of significance was obtained, then the increased level was quoted (eg. $p<0.001$).

Scatter plots were drawn throughout this study as an aid to deciding the most appropriate statistical test to apply to the data. Due to the large number of parameters investigated (41) and the complexity of the possible inter-relationships between these parameters, not all plots have been presented. In place of numerous plots, tables presenting summarizing statistics of several plots and analyses were produced. Bold print in these tables indicated statistically significant results. To reduce the complexity of the analyses and to aid comparisons, the methods used to investigate the various parameters were repeated in different experiments.

4.7.1 Parametric analyses

Tests for normality

Two simple graphical procedures were employed to determine the normality of the data distributions. Both methods required an arbitrary estimation of the extent of any departure from normality, based on the graphical distribution of the data. While care was taken to determine the normality of the data, the procedure has been shown by Ryan *et al* (1985) not to be crucial, because they suggest that techniques based on the normal distribution give very good, though approximate, answers even if the data is not normal.

Firstly histograms were drawn of the data and the resulting distribution compared with the standard bell-shaped normal distribution. Secondly, the "nscores" procedure in Minitab (Ryan *et al*., 1985) was used to produce a line, the linearity of which was correlated with the normality of the data.

Correlation analysis

The correlation coefficient (r) shows the extent of correlation between two variables and quantifies the scatter of the points around a linear regression line. A value of 0 indicated that the two parameters were not at all correlated and a value of 1 indicated complete

correlation. An explanation of the calculation of **r** and graphical examples of different values of r are given by Campbell (1974). **r** was calculated throughout this study using Minitab (Ryan et al., 1985). Care was taken to ensure that the two sets of data to be correlated were independent.

Linear regression analysis

The main method employed to quantify the relationship between, or influence of, one parameter on another was the regression analysis (Snedecor and Cochran, 1972; Zar, 1974). This analysis was used to calculate the equation for the line of best fit, on a scatter diagram, of pairs of variables. The method is only valid when: the distribution of values of y for a fixed value of x is normal; and the relationship between the two variables is linear. The derived equation describing the line of best fit was in the form:

$$y = mx + c$$

where: m = the slope of the line

c = the intercept on the y axis

The degrees of freedom, significance level and correlation coefficient were always shown with this equation. Linear regression analysis was calculated throughout this study using Minitab (Ryan et al., 1985).

Transformations

The linear regression equation, explained above, describes the straight line of best fit. The above equation will not apply, or will not be accurate, if the variables are not related linearly. Under certain circumstances one or both variables will require transformation to produce a linear relationship. This procedure was used particularly when an accurate regression equation was required, to compare with another equation or to use in predictive statistics. Examples of transformations, such as logarithmic, square roots, and inverses, are shown in Ryan *et al*. (1985). A trial and error method was used to determine the most appropriate transformation, as indicated by the straightness of the line of best fit and the degree of correlation.

Student's t-tests

Three analyses using the t-distribution were employed during this study. A clear description of the use of t-tests is given by Campbell (1974, p 144 - 162). The various t-tests were calculated throughout this study using Minitab (Ryan *et al*., 1985). The analysis was used to obtain an interval estimate of a single population, indicating the 95 % confidence limits of the mean.

A two-sample t-test was used to compare the means of two populations. The null hypothesis of the analysis was

that the two population means were equal. The procedure effectively calculated whether the interval estimate for the difference of the two means included zero.

A third procedure used frequently in this study was the paired-sample t-test. This procedure was used to determine an interval estimate of the mean difference between pairs of data and to calculate a significance test that this difference was zero. An example of the use of this test in the present study was the investigation of the influence of pigmentation on the mean weights of fish held at different depths.

Analysis of variance

One-way analysis of variance (ANOVA) was employed in this study to compare the means of more than two normally distributed populations, as described by Campbell (1974, p 177 - 205). This test was used in place of multiple t-tests, comparing pairs of variables, because the error associated with the t-test increased with the number of comparisons. The ANOVA procedure determined the extent of variation of the data which was due to population differences and to random variation. The null hypothesis, that the population means were the same, was rejected when the variation due to population was much larger than random error. The ANOVA was calculated throughout this study using Minitab (Ryan et al., 1985).

4.7.2 Nonparametric analyses

Sign tests

The sign test (Ryan et al., 1985) uses a binomial distribution to calculate the probability that a variable was either less or greater than the median. Assumptions, which are made in parametric tests, are not made about the shape of the population distribution. The sign test is similar to the t-test because the sign procedure can be used to calculate the confidence interval of the median, rather than the mean, and uses the interval to test if another population median is significantly different.

Mann-Whitney

The Mann-Whitney procedure (Ryan et al., 1985) is the nonparametric analogue of the t-test. The procedure involved the combination of the two sets of data, which were ranked. The sum of the ranks of one population (W) was calculated. A confidence interval about the median difference between the two variables was also calculated.

If zero was included in the confidence interval then there was no significant difference in the medians.

CHAPTER 5

HYDRODYNAMIC ASPECTS PART 1

RESIDENCE TIME STUDIES

5.1 INTRODUCTION

A fish tank can be considered as a flow-through chemical reactor in which reactants, such as food and oxygen, are converted by the fish into new tissue. By-products such as oxygen-depleted water, CO_2 and nitrogenous metabolites result. Reactants must be distributed throughout the vessel if the maximum use of space is to be achieved and the conversion of reactants into products is to be conducted most efficiently. Chemical mixing is however one of the most intractable of all unit operations in chemical engineering (Cholette and Cloutier, 1959).

Such principles are not new to the chemical engineering industry and much information is available in the literature regarding reactor designs, development, uses and theory. Much of this information is beyond the scope of this study but certain aspects are pertinent to fish tank design.

There are two main mixing processes: batch and continuous. In the batch system the state of the process is changing with time, while in continuous reactions a stationary state is built up at each point in the system,

which remains unchanged for as long as the flow rates remain constant. The latter type of reaction is extended in space rather than time (Denbigh, 1944). Flow through and recirculating aquaculture systems are effectively continuous reactors.

Weatherley (1983) states that in aquaculture, little has been done to study the overall dynamic characteristics and that mathematical modelling is a flexible tool for operators which will improve design. Senecal (1957) explains the need for a study of fluid mechanics in process industries.

Three types of analysis of hydrodynamic parameters are particularly suitable for studying aquaculture tanks: residence time distribution, flow velocity determination and flow visualization. The latter two methods and a comparison of all three methods will be described in following chapters, whilst this chapter will deal primarily with the study of residence times.

5.2 OBJECTIVES

The two main objectives of this study were:

1. To investigate the effect of variations in tank management, such as water depth, flow rate and stocking density of turbot, on hydrodynamic characteristics, such as residence time, short-

circuiting, mixing efficiency and dead volume.

2. To compare these hydrodynamic parameters of tank mixing with biological and water quality data obtained under the same maintenance regimes, and so investigate any correlations between them.

5.3 THEORY

5.3.1 Reactor hydrodynamics

There are many different mixing conditions within continuous flow systems, ranging from complete, or perfect mixing, to piston or plug flow, as described by Dankwerts (1953). Fluid which is perfectly mixed has uniform properties which are identical to the effluent stream. Piston flow refers to a fluid which passes through a vessel with a constant and equal velocity on a parallel path. In practice either extreme situation is almost impossible to achieve due primarily to longitudinal mixing, molecular or eddy diffusion and viscous effects in Newtonian fluids. In practice a combination of complete mixing and piston flow occurs.

Short-circuiting is an important aspect of mixing determinations. The term is however ambiguous because a tracer may rapidly appear at the effluent because it has either been mixed quickly or has channeled to the outlet with little mixing. Short-circuiting may therefore occur

in both well mixed and badly mixed vessels. Cholette and Cloutier (1959) suggest the term should refer only to the poor mixing situation. Denbigh (1944) defined this mathematical by-passing as the loss of reactants within a shorter time than is required to complete the reaction. Similar descriptions were given by MacMullin and Weber (1935) and Weber (1953). Aquaculturists Burrows and Chenoweth (1955) simplified it as: water which passes quickly from the inlet to the outlet without circulating to all parts of the pond. In aquaculture short-circuiting will result in oxygenated water leaving the tank prematurely.

"Hot spots" (Haskell et al., 1960), pockets or dead areas can build up within reactors, particularly those where short-circuiting is prevalent. These regions have been described by several authors including Burrows and Chenoweth (1955) studying rectangular raceways, Wheaton (1972) and Larmoyeux et al. (1973) primarily describing circular fish tanks and Burrows and Chenoweth (1970) developing rectangular circulating ponds. In these areas, which are by-passed by the main flow and so receive little circulation, a build-up of reactants results in an overall uneven distribution of reactants and by-products throughout the vessel. The effective working volume of the tank will be reduced and so will be less efficient than expected, in terms of the conversion of reactants to products.

Rosenthal et al. (1982) studying fish tanks found that these dead areas contained high NH_3 levels and low DO concentrations. Fish may avoid these dead areas and this may lead to stocking densities in the rest of the tank which are higher than expected. The usable volume of the tank will be less than that anticipated, so theoretical maximum stocking densities may in practice be exceeded. Alternatively fish may enter the dead areas, due to overcrowding, hierarchies or because they are less fit to compete for the alternative space. These fish will then be in sub-optimal water quality conditions and so may become prone to disease and reduced growth rates, as was indicated by Rosenthal et al. (1982). It is therefore important to eliminate dead areas in culture tanks if optimum conditions are to be approached, and parameters within the tank, such as stocking density, are to be quantified accurately. Klapsis and Burley (1984) gave a clear account of the hydrodynamic problems associated with the design of aquaculture tanks.

Several other terms described by Shapiro (1961) and Carstens (1968) are relevant to the design of aquaculture tanks. Skin friction is the tangential force or drag exerted by a fluid flow on a perfectly smooth surface. A boundary layer is a slower moving or static fluid layer near the surface of a vessel such as a pipe or tank wall. Form drag is the force which disturbs streamlines and is caused by objects obstructing a flow, such as culture animals. The Reynolds Number refers to the dynamic

similarity of geometrically similar bodies in a fluid. This is an important parameter to consider when scaling up from pilot plants. Scale-up problems are discussed by Weber (1953) Danckwerts (1953) and Burrows and Chenoweth (1955). The transition from laminar to turbulent flow in biological systems is discussed by Leyton (1975).

5.3.2 Experimental determination of fluid mixing in reactors

Comprehensive accounts of the methods available for studying fluid mixing in closed vessels are given by Levenspiel (1966 and 1972). The former publication gives a worked example of a standard exit age distribution method, and so may be most useful to researchers from other fields. Fluid mixing within vessels, even of a simple design and in a steady state, is extremely complex. Methods giving a general picture have however been proposed. These methods investigate the length of time that each particle of fluid spends in the vessel. The time spent by each particle in the reactor is its residence time. The distribution of residence times of the fluid can be obtained and used to estimate mixing.

In one such method, the stimulus-response technique, the flow in the vessel is disturbed and the response studied. Tracers, used primarily in this context to study the response, can be injected into the system in four ways: randomly, cyclically, a step input and a pulse

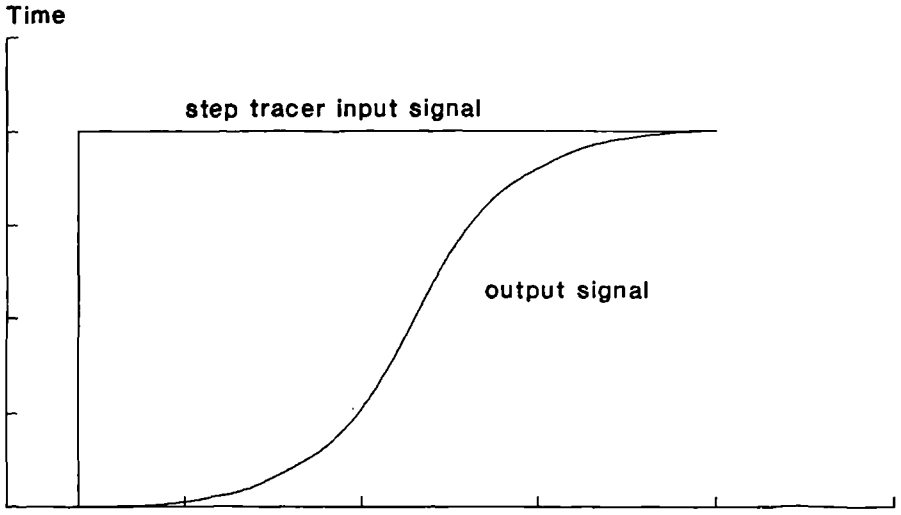
input. The latter two stimuli are the most simple to quantify and are described in Figure 5.1.

The injection of a constant concentration of tracer as a step input into the influent stream (Figure 5.1a), will cause the effluent stream tracer concentration to rise from zero to the injection concentration. Plotting this type of response against time is called an "F-curve" (where F is the fraction of the reaction) as described by Danckwerts (1953) and Levenspiel (1966). Analysis using a step input has been used by Cholette and Cloutier (1959) and MacDonald and Piret (1951) studying stirred reactors and by Bernard and Wilhelm (1950) studying diffusion through packed beds.

Alternatively a pulse input can be injected into the vessel and the change in effluent tracer concentration with time can be studied (Figure 5.1b). This type of response produces a "C-curve" as described by Danckwerts (1953) and Levenspiel (1966). Pulse inputs have been used by Burley and Klapsis (1985) studying fish tanks. Several studies, including Burrows and Chenoweth (1955) comparing different fish tanks and Hughes et al. (1974) in developing a lobster rearing tank, do not clearly state which method was employed, though in the case of these two investigations which are particularly relevant to this study, it is thought that a pulse input was used.

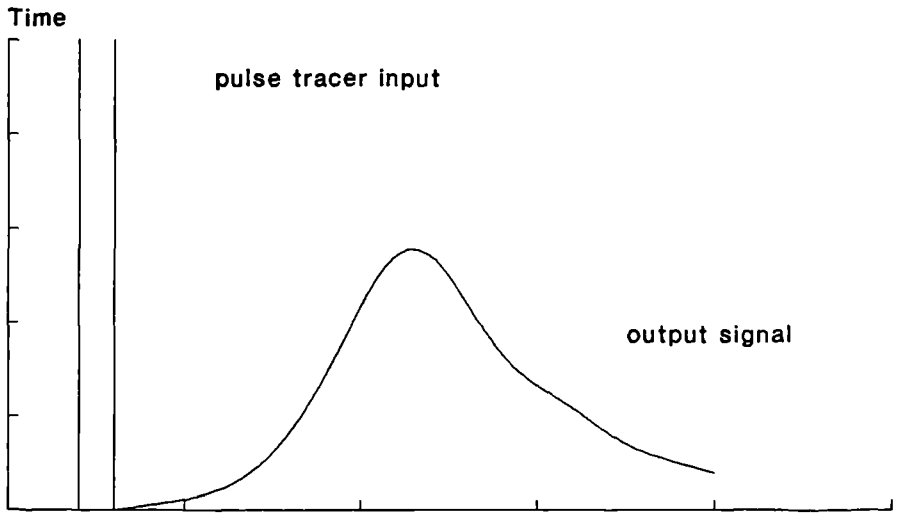
From the change in tracer concentration downstream of

Figure 5.1a: Stimulus response techniques commonly used in the study of the behaviour of systems - F-curve



After Levenspiel (1966)

Figure 5.1b: Stimulus response techniques commonly used in the study of the behaviour of systems - C-curve



After Levenspiel (1966)

the injection point, the distribution of residence times for the particles in the fluid is obtained and an "E-curve" constructed, as described by Levenspiel (1966). E-curves in which both the age of the particles leaving the vessel and time are expressed in dimensionless units, can be used to compare different designs of tank. Examples of dimensionless E-curves are presented by Burrows and Chenoweth (1955) and Burley and Klapsis (1985) both investigating mixing in fish tanks.

It was decided to use the pulse signal E-curve procedure rather than a step signal method for three reasons:

1. The method of analysis appeared to be simpler.
2. It was considered beneficial to minimize the volume of tracer entering the tanks so minimizing stress to the fish.
3. Less wastage of water in the recirculation culture system, because water containing dye tracer had to be run to waste (see section 5.4.4).

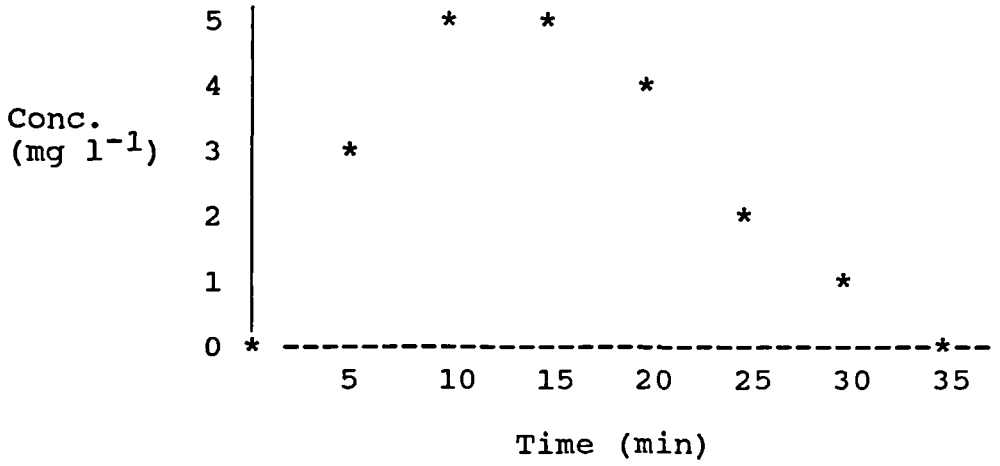
Example of E-curve construction

To clarify the analysis, an expansion of the worked example in Levenspiel (1966) is presented. Table 5.1 and Figure 5.2 shows the concentration of tracer (C) measured downstream of a pulse input of tracer at **t** min after injection.

Table 5.1: Variation in effluent tracer concentration with time

Time t (min)	Tracer concentration (mg l^{-1})
0	0
5	3
10	5
15	5
20	4
25	2
30	1
35	0

Figure 5.2: Variation in effluent tracer concentration with time (E-curve)



The area under the curve on integration is equivalent to the total amount of tracer added. This is calculated as the product of concentration at each sample time (which is in practice instantaneous) and the time over which the sample was estimated to have occurred, ie. the interval between samples. The integral is actually the area of a histogram describing Figure 5.2:

$$Q_t = C_1\,dt + C_2\,dt + C_3\,dt \ldots\ldots\ldots C_t\,dt = \hat{E}Cdt$$

where:

Q_t = quantity of tracer added

C_t = tracer concentration at time t

dt = change in time = interval between samples

$\hat{E}C$ = sum of tracer concentrations

in the above example:

$$\underset{(\text{mg } l^{-1})}{} \qquad \underset{(\text{min})}{}$$

$$Q_t = (3 + 5 + 5 + 4 + 2 + 1)\ 5 = 100 \text{ mg min } l^{-1}$$

The axes are converted into dimensionless units to enable the comparison of different experimental regimes and tank designs. Tracer concentrations are expressed as a proportion of the total, Q_t.

$$E_{(t)} = \frac{C}{Q_t}$$

where:

C = tracer concentration

$E_{(t)}$ = proportion of total tracer exiting at time t

In the above example:

t	0	5	10	15	20	25	30	35
$E_{(t)}$	0	0.03	0.05	0.05	0.04	0.02	0.01	0

For example, at 5 min, 0.03 of the total tracer

injected exited the reactor per minute.

Similarly, in standardizing, time is converted to dimensionless residence time θ by expressing it as a proportion of the mean residence time t_m:

$$t_m = \frac{\hat{E}Ct}{\hat{E}C}$$

In the example:

$$t_m = \frac{(5x3)+(10x5)....+(30x1)}{3 + 5 + 5 + 4 + 2 + 1} = \frac{300}{20} = 15 \text{ min}$$

$$\theta = \frac{t}{t_m} = \frac{t}{15}$$

To convert $E_{(t)}$ into E:

$$E_{(t)}\ t_m = E$$

In the example:

θ	0	0.33	0.67	1.00	1.33	1.67	2.00	2.33
E	0	0.45	0.75	0.75	0.60	0.30	0.15	0

A difference has arisen in the literature as to the exact scaling of the y axis of the E curve. It is likely that this difference has occurred because of an alteration in the worked example described by Levenspiel in the two editions of his book (Levenspiel 1966 and 1972). The example shown above from the former edition shows E, expressed as $E_{(t)} * t_m$, plotted against θ. The latter edition does not include this final stage in the calculation of E and shows a plot of E ($E_{(t)}$ in the previous edition) verses θ.

Investigation of both the method description and the

magnitude of the y axis scale indicate that E and $E_{(t)}$ have been interpreted differently. Burrows and Chenoweth (1955) appear to have conformed to the former method while Burley and Klapsis (1985) have used the latter method. Neither method produces incorrect results, but when comparing data, differences in the range of the y axis, but not the shape of the curve will be apparent. The former method has been used throughout in this study because $E_{(t)}$ can not be considered as dimensionless as it is expressed as a proportion per minute. It should be noted that the y axis should not be greater than unity as stated by Burley and Klapsis (1984) if $E_{(t)}$ is used. Values may however exceed 1 if E as $E_{(t)} * t_m$ is used.

5.4 MATERIALS AND METHODS

5.4.1 Tracers

To perform the exit age distribution analysis a tracer was required as the pulsed signal.

Properties required

1 Concentration easily determinable.

2. Chemical and U.V. stable.

3. Similar specific gravity to test fluid, ie. fresh and seawater, if diluted appropriately.

4. Inexpensive.

5. Non-toxic.

6. Non-adhesive.
7. Reliable calibration.
8. Reliable availability.
9. Visually obvious, for flow visualization studies.

Tracers used in previous studies

Many different tracers have been used to study mixing within vessels and for flow visualization. Salt solutions have proved popular. MacDonald and Piret (1951) proposed the use of "brine" for studying stirred reactors. Cholette and Cloutier (1959) mixed in a 0.05N solution of sodium chloride to a vessel and then added a freshwater supply. Klapsis (1983) and Burley and Klapsis (1985) injected sodium chloride solution into fish tanks and measured the concentration with a conductivity meter attached to a chart recorder. Von Rosenberg (1956) added sucrose to increase the viscosity of two different concentrations of sodium chloride to calculate the displacement curves of fluid in a sand bed. Advantages to using salt solutions are that they are inexpensive, non-toxic at low concentrations and are easy to analyse. They are however unlikely to have the same specific gravity as the background fluid and are not visually apparent, so could not be used for flow visualization studies.

Denbigh et al. (1962) and Danckwerts and Wilson (1963) used a starch-iodine colourimetric reaction to measure the age of the effluent fluid. This method may be

toxic to animals present and is more expensive than salt tracer methods.

Rhodamine B has been successfully used to study mixing in aquaculture tanks by Hughes et al. (1974) and Rosenthal et al. (1982). This tracer does fulfill many of the criteria described above and may be suitable for this study.

Fox and Gex (1956) placed a phenolphthalein solution, which appears red at pH 7.9-8.1, into a tank and added 2N sodium hydroxide until a red colour was produced. An influent stream of 2N hydrochloric acid was then introduced and the extinction of the colour measured as a indicator of mixing. This method is clearly inappropriate for aquaculture purposes for reasons of toxicity.

Gentian violet crystals placed at strategic points in fish tanks by Burrows and Chenoweth (1955) were used to visualize fluid flows, but would be difficult to adapt to hydraulic mixing studies. Another common dye, methylene blue, was used by Bernard and Wilhelm (1950) to study turbulent diffusion through packed beds.

Milk, as suggested by Vogel and LaBarbera (1978) is a commonly used tracer. Whilst milk is non-toxic cheap and commonly available it has a tendency to adhere to tank sides forming a film which causes a decline in tank hygiene.

Less common tracers include potassium permanganate (Chamberlain, 1976), photoreactive dyes (Goldish et al., 1965), suspended plastic particles (Vogel and Feder, 1966) and small particulates such as carmine, graphite, clay and starch (Crisp and Southward, 1956). Each of these are either expensive or difficult to ensure mixing is not biased by density differences between the tracer and the carrying fluid.

Overall, of the methods discussed, rhodamine B appeared most suitable to this study, however its toxicity was in doubt, so novel tracers were examined.

Tracers used in this study

A food colouring suitable for human consumption, which could be visualized and analysed in low concentrations as well as fulfilling the criteria listed above, was considered appropriate. Preliminary tests were undertaken to assess its suitability for hydrodynamic mixing studies and flow visualization. For visualization work a dark colour such as blue or green was preferable to red or yellow.

Three dyes were tested:

1. (SCB) - "Supercook blue food colouring", costing 29p for 38 ml from Wm Low Supermarkets consisted of water, glycerine, brilliant blue FCF, E122 Carmosine and preservative E210.

2. (COB) - "Co-op blue food colour" costing 21p for 28 ml from Co-op Stores consisted of iso-propyl alcohol, brilliant blue FCF, Panceau 4R (E124) and acetic acid.
3. (COG) - "Co-op green food colour" costing 21p for 28 ml from Co-op Stores consisted of iso-propyl alcohol, tartrazine,(E102), green S (E142) and acetic acid.

All three dyes were observed to dissolve completely in both sea and fresh water, with no particulates or precipitates. Densities were approximately equivalent to the fluid in which they were diluted. Serial dilutions of the dyes were made up in distilled water and the concentration ranges detectable on a spectrophotometer were determined. The absorbance of dyes SCB and COB were determined at 630 nm and the absorbance of dye COG at 550 nm. SCB and COG were detected to 0.01 ml l^{-1} while COB was detected to 0.001 ml l^{-1}. COB showed the highest absorbance levels for each concentration and so was used during this study.

To determine the wavelength of maximal absorption, a 1 ml l^{-1} concentration of COB in distilled water was analysed at 2 nm intervals in the range 620 - 640 nm. Maximal absorption occurred at 630 nm, coincident with the maximal absorption wavelength of 630 nm for brilliant blue FCF (Windholz, 1983).

Two curves calibrating light absorbance to dye concentration were constructed (Figure 5.3) using serial dilutions of COB in tap water and in approximately 35 ppt salinity seawater taken from the experimental recirculation system. Methods and apparatus used for calibration and experimental determination of absorbance were the same. The linear regression equations within the range 0.005 - 0.5 ml l^{-1} were:

Freshwater: $y = 5.1x10^{-4} + 0.938\ x$

$r=1.000$ $df=5$ $p<0.001$

Seawater: $y = 1.79x10^{-3} + 0.872\ x$

$r=1.000$ $df=5$ $p<0.001$

where:

y=absorbance x=dye concentration (ml l^{-1})

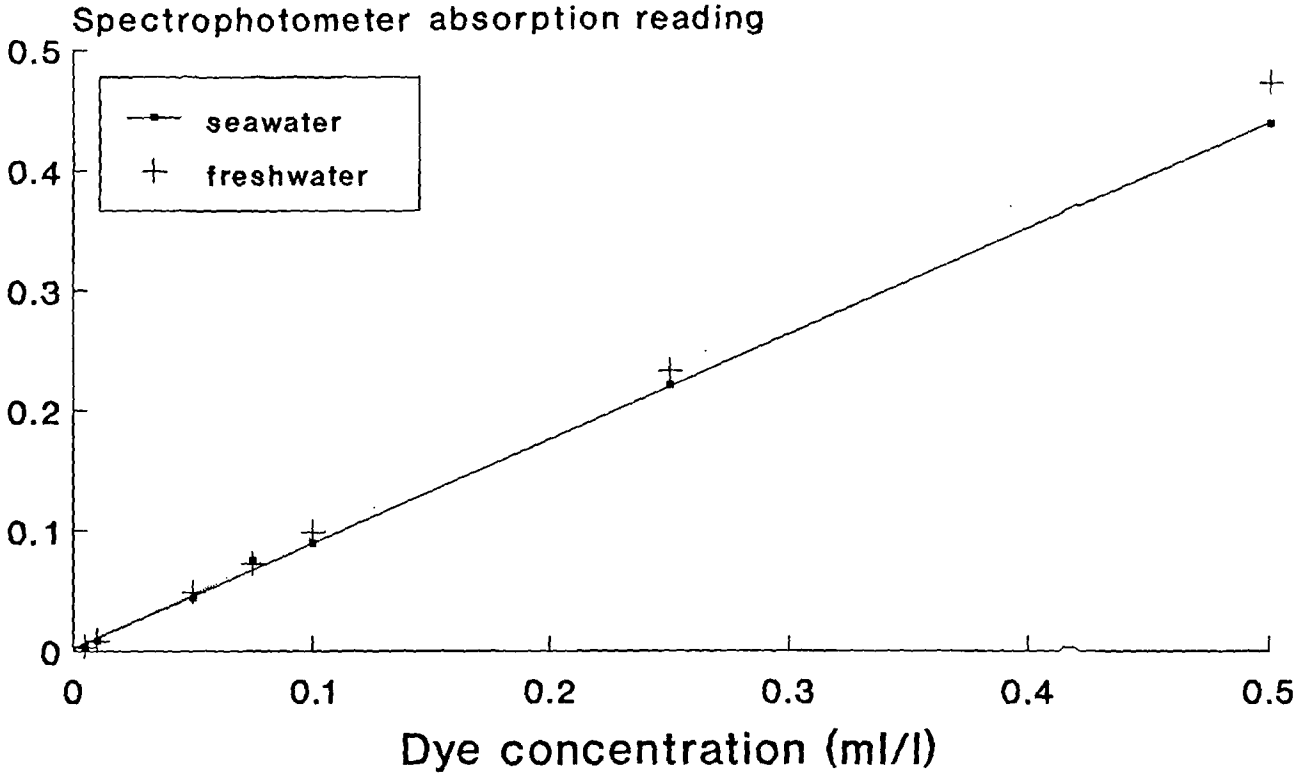
Ideally the regression lines should pass through the origin assuming the water contains no other constituents which absorb at 630 nm. The "a" values in the equations approximated 0, and the association between absorption and dye concentration was linear, so the following simplified equations for converting absorbance to dye concentration were assumed and used in future calculations.

Freshwater: $y = 0.938\ x$

Seawater: $y = 0.872\ x$

In practice dye concentrations used during the experiments were at the lower end of the calibration

Figure 5.3: "COB" dye tracer calibration curves



FW: y=0.001+0.938x, df=5, r=1.000, p<0.001. SW: y=0.002+0.872x, df=5, r=1.000, p<0.001.

scale, so the influence of salinity was negligible.

The approximate quantity of dye required for each experimental run was estimated. The volume of a 15 cm deep tank was calculated as 30.9 l, and the detectable range of the dye was approximately 0.001 - 1.0 ml dye l water^{-1}. Burrows and Chenoweth (1955) suggested that the maximum tracer concentration in a tank was approximately equal to the concentration if uniformly dispersed. An instantaneous injection of tracer was assumed and, allowing some margin for error, a maximum concentration of 0.08 ml dye l water^{-1} was required, the following volume of dye was needed for each test:

$$0.08 \text{ ml } l^{-1} \times 30.9 \text{ l} = 2.47 \text{ ml dye test}^{-1}$$

Though highly inaccurate, this estimate of dye quantity was adequate and so was used in all residence time experiments in this study. To ensure the densities of the injection fluid and the treatment tank water were similar, the dye was diluted to 10 ml with either fresh or seawater, depending on the background water salinity.

5.4.2 Apparatus design

The design of all tanks, pipes and treatments used during other experiments, with which hydrodynamic data was to be compared, was retained. Minor adaptations were made for redundant equipment, a sample point and dye injection point. The recirculation system was set up as shown in

Figures 5.4 and 5.5, depending on whether seawater or freshwater was used in the treatment tanks.

In addition to recirculation system equipment, apparatus for determining residence time distributions in fish tanks was used. The apparatus consisted of a Cecil Instruments CE373 linear readout grating spectrophotometer; Servoscribe 1s potentiometric chart recorder; Glen Creston variable speed, twin-head, peristaltic pump; brass gas tap fittings as sample and injection ports; 50 ml syringe; 1 ml flow-through semi-micro spectrophotometer cell; silicon tubing; COB tracer (section 5.4.1); and stop clock, set up as shown in Figure 5.6.

The injection port was located 25 cm above the inlet spray bar so that it was always above the water level, but its distance from the spray bar was minimised. This minimised the time delay from dye injection to first appearance in the tank. The port, adapted from a brass gas fitting, was screwed into the tank inlet pipe. A syringe containing dye was connected to a hollow tube attached to the outside of the port, for injection of the tracer into the inlet pipe. The sample port was a brass gas fitting screwed into the outlet standpipe as close to the outlet collection pipe as possible. This location minimised the time between the exit of the dye from the tank and its determination at the spectrophotometer and mixing effects due to the tank outlet pipe were reduced.

Figure 5.4: Flow diagram of experimental system - seawater (not to scale)

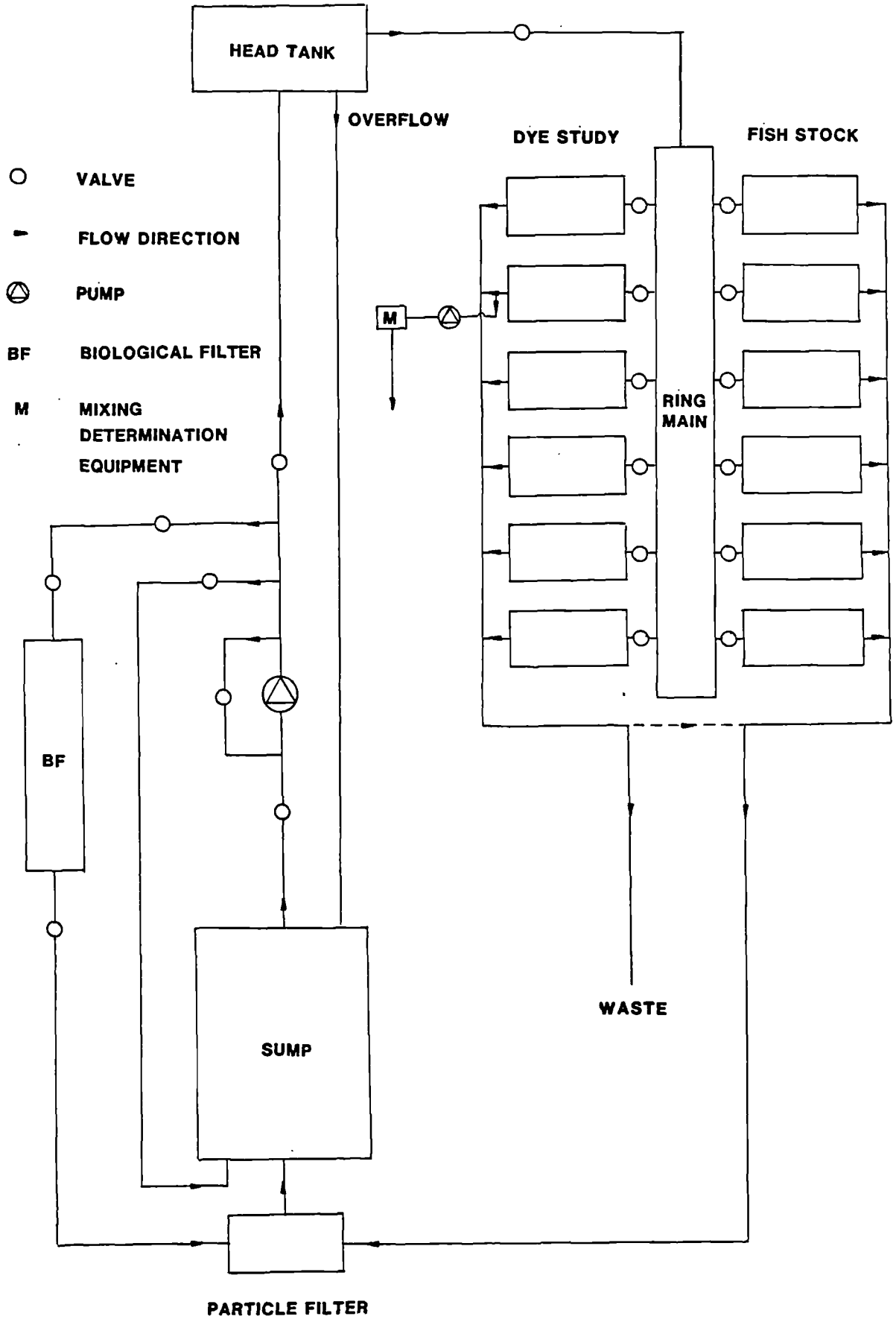


Figure 5.5: Flow diagram of experimental system - freshwater (not to scale)

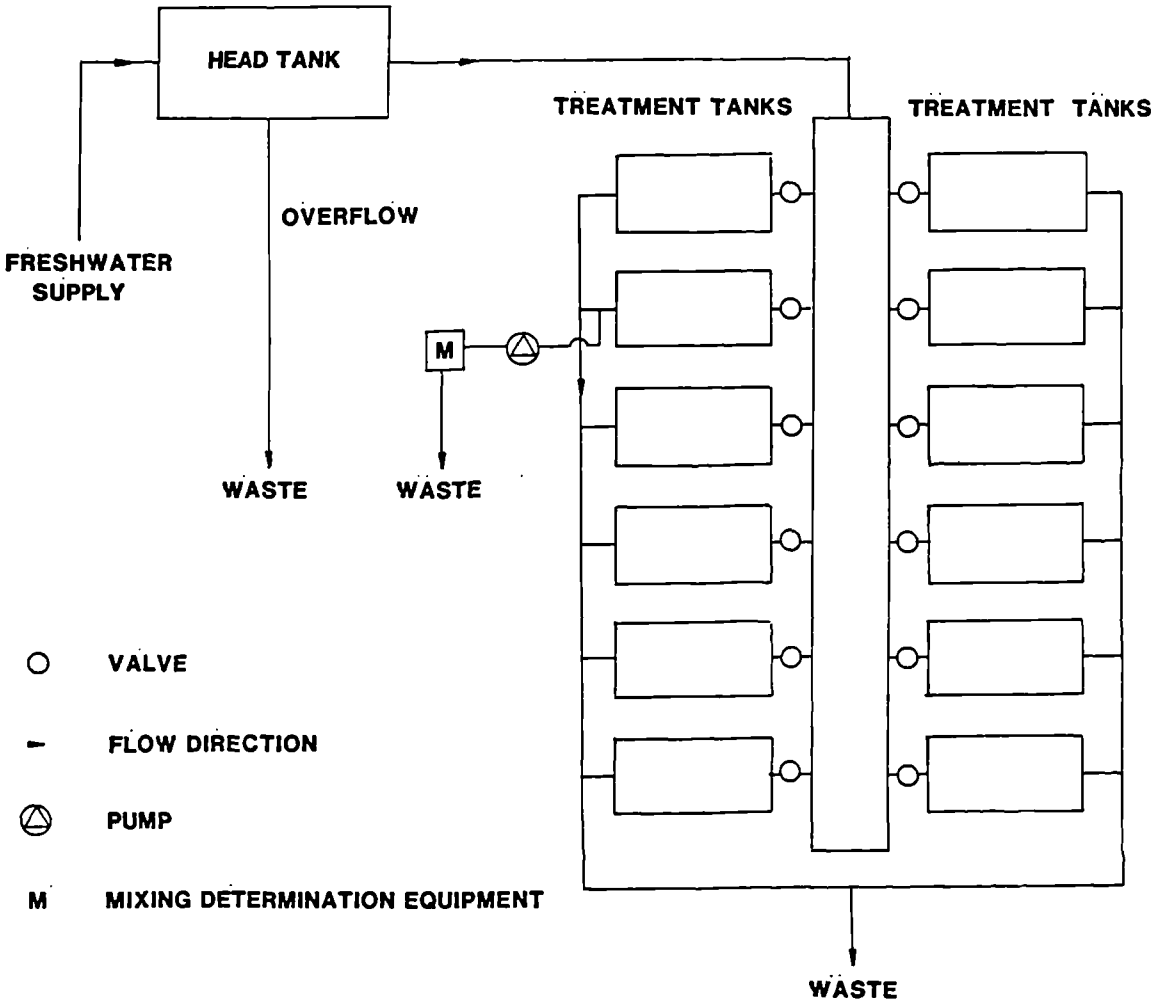
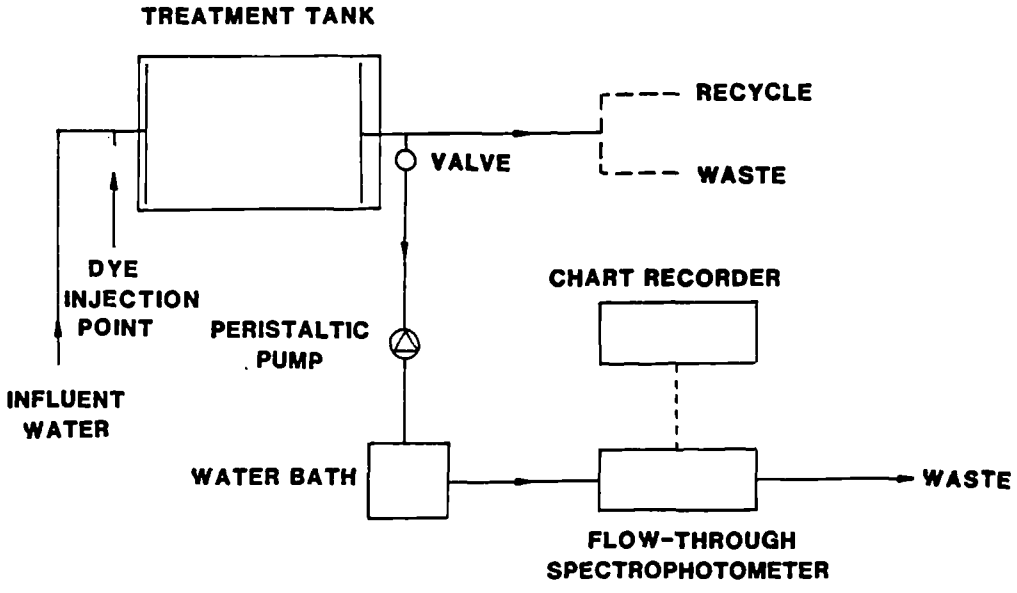


Figure 5.6: Flow diagram of mixing determination equipment (not to scale)



Silicon tubing connected the sample point, peristaltic pump, spectrophotometer and waste bucket. It was also important to keep the length of tubing between the sample point and spectrophotometer cell as short as possible to reduce mixing effects due to the tubing and to minimize the time lag between tracer exiting the tank and its determination. It was realised that a peristaltic pump would mix the fluid in the tubing, however the rate of change of absorbance readings was rapid during periods of rapid change in tracer concentration at the tank outlet, particularly noticeable at first response. This indicated that longitudinal mixing was not a great source of error. The same pump and pump speed was maintained throughout the experiment, so any error due to longitudinal mixing was at least kept constant. In retrospect a gravity flow of fluid through the spectophotometer cell without a pump may have been preferable, but may have introduced other technical problems such as maintenance of an adequate, stable flow. Tubing which extended out of the spectrophotometer was permanently attached to the spectrophotometer cell. Tube unions for attachment to the tube from the pump and to waste were located outside of the spectrophotometer housing. Disturbance to the cell during coupling and uncoupling of tubing was therefore minimised.

5.4.3 Fish stock

Juvenile post-weaned turbot used in the mixing study

experiments were obtained from Clearwater Aquaculture (International) Limited, Isle of Man, in April 1989. The wet weight, length and pigmentation of the fish at the beginning and end of the experiments are shown in Tables 5.2 and 5.3.

5.4.4 Experimental method

To minimise the background dye concentration in the recirculation system, dye introduced into the treatment tank was not permitted to remain within the system. Water containing tracer in the tank effluent and spectrophotometer effluent were run to waste. A sizeable proportion of the recirculation system water was therefore discarded during the experiment. Due to limitations in the supply of seawater, freshwater was used for the majority of runs. Mixing differences, due to salinity variations were considered negligible. An important consequence of the use of freshwater was that turbot could not be introduced into the treatment tank. It was expected that the turbot shape and movement would influence the flows in the tank. A compromise method was achieved. Though previous studies (Burrows and Chenoweth, 1955; Hughes *et al.*, 1974; Burley and Klapsis, 1985) did not indicate that duplicate runs were carried out, it was expected that some random variation in mixing would occur. Triplicate mixing study treatments were analysed in tanks containing freshwater and no fish. One run of each treatment using seawater, with the appropriate stocking

Table 5.2: Initial dimensions of stock turbot used in mixing study experiments

No.	length (mm)	weight (g)	pigm. p/m	No.	length (mm)	weight (g)	pigm. p/m
1	76	8.9	m	26	61	4.4	m
2	60	3.9	m	27	79	9.7	m
3	73	6.9	m	28	75	6.9	m
4	73	7.6	m	29	65	5.4	m
5	71	7.5	p	30	84	12.3	m
6	70	6.4	m	31	75	7.4	m
7	63	4.7	m	32	72	6.8	m
8	64	5.1	m	33	63	4.6	m
9	69	6.6	m	34	61	4.2	m
10	80	9.8	m	35	83	10.8	m
11	68	5.6	m	36	75	8.0	m
12	74	7.8	m	37	75	7.9	m
13	73	7.5	m	38	72	7.1	m
14	69	6.0	m	39	71	6.6	m
15	57	3.6	m	40	58	3.4	m
16	65	5.2	m	41	73	7.7	m
17	76	8.5	m	42	66	5.3	m
18	67	5.0	m	43	62	4.9	p
19	74	7.8	m	44	71	6.3	m
20	73	8.3	m	45	61	3.9	m
21	62	4.7	m	46	76	8.2	m
22	58	3.9	m	47	54	2.8	m
23	76	8.2	p	48	69	6.5	m
24	59	3.5	m	49	65	5.1	m
25	58	3.9	m				

p = pigmented m = malpigmented

Total initial weight = 313.1 g

Mean initial weight = 6.4 g

Table 5.3: Final dimensions of stock turbot used in mixing study experiments

No.	length (mm)	weight (g)	pigm. p/m	No.	length (mm)	weight (g)	pigm. p/m
1	84	10.5	m	26	89	11.4	m
2	75	6.9	m	27	79	8.9	m
3	71	6.7	m	28	86	12.8	p
4	81	9.9	m	29	72	6.6	m
5	79	8.1	m	30	66	5.1	m
6	70	5.6	m	31	64	5.2	m
7	83	10.4	m	32	83	9.4	m
8	85	10.7	m	33	82	9.7	m
9	81	9.0	m	34	80	9.5	m
10	78	7.8	m	31	85	10.0	m
11	76	7.6	m	36	67	4.9	m
12	86	11.3	m	37	72	6.4	m
13	75	6.7	m	38	90	12.8	m
14	79	7.7	m	39	82	8.7	p
15	85	10.4	m	40	68	5.9	m
16	62	3.9	m	41	75	6.8	m
17	89	13.6	m	42	92	14.9	m
18	73	6.9	m	43	77	8.4	m
19	85	10.3	m	49	73	6.8	m
20	79	8.4	m	45	75	7.3	m
21	75	7.9	m	46	65	4.5	m
22	80	8.4	m	47	82	9.3	m
23	67	4.7	m	48	80	8.7	m
24	85	9.4	m	49	70	5.5	p
25	72	6.9	m				

p = pigmented m = malpigmented

Total final weight = 409.2 g

Mean final weight = 8.4 g

Initial and final mean weight = 7.4 g

N.B. Initial and final fish numbers do not necessarily refer to the same fish.

density of fish present, was also analysed to investigate if triplicate freshwater results were representative of the seawater situation. Differences in mixing results between fresh and seawater must then have been due to either: random variation; change in water medium; or the presence of fish. Table 5.4 summarises the residence time analyses carried out. Approximately 2500 l of seawater was run to waste in this experiment.

Freshwater experiments

A 50 ml graduated syringe containing 2.47 ml of tracer made up to 10 ml with freshwater was attached to the injection port. Air was drawn into the port by a Venturi effect, ie. the movement of the influent water flow, when no syringe was attached, causing bubbles and disruption of the normal tank mixing. The sample was therefore injected a minimum of 10 min after attachment of the syringe, to allow tank mixing to stabilise. The port was left open for as short a time as possible. Before each run the spectrophotometer was standardized using system water with no dye present. The output from the peristaltic pump was set at a constant 47 ml min^{-1}. The chart recorder paper speed was set to a high value so that large scale changes occurring near the beginning of the run were distinguished and so that first response time and time to maximum concentration were measured accurately.

At the beginning of an experimental run the stop-

Table 5.4: Summary of residence time analyses

water depth (cm)	flow rate (l min^{-1})	residence time (min)	stocking density (fish $tank^{-1}$)
18	2.0	18.54	10
			20
15	2.0	15.45	10
12	2.0	12.36	10
			13
9	2.0	9.27	10
			10
6	2.0	6.18	10
			7
3	2.0	3.03	10
			3
18	3.4	10.80	10
			20
15	2.9	10.80	10
12	2.3	10.80	10
			13
9	1.7	10.80	10
6	1.1	10.80	10
			7
3	0.6	10.80	10
			3
9	0.5	37.08	10
9	1.0	18.54	10
9	2.0	9.27	10
9	4.0	4.64	10
9	8.0	2.32	10
9	13.0	1.43	10
9	2.0	9.27	50
9	2.0	9.27	40
9	2.0	9.27	30
9	2.0	9.27	20
9	2.0	9.27	10
9	2.0	9.27	5
9	2.0	9.27	0

clock was started and the tracer was injected into the influent stream. To ensure continuity 1 ml of tracer solution was injected each second for the first 10 s of the experiment. Observations were made of the mixing of tracer in the tank until the dye was an homogeneous mix with the water. A note was made on the chart paper 1 min after the start of the experiment, so that the exact time of the start of the experiment could be determined (see calculations section). After the peak in tracer concentration had passed or at least 2 min from the beginning of the experiment, the chart paper speed was slowed, because changes in tracer concentration were then usually less rapid and more uniform. Absorbance was measured until values returned to zero, usually within 90 min. Triplicate runs for each treatment were carried out.

Seawater experiments

The freshwater experimental method described above, was repeated, but with the following modifications. 2.47 ml of dye was diluted to 10 ml with recirculation system seawater. Prior to injection of the tracer, the tank and spectrophotometer streams were diverted from recirculation to waste, and returned to recirculation as absorption values approached zero near the end of the experiment. The appropriate number of turbot were transferred to the treatment tank at least 30 min before the start of each experimental run and allowed to settle

with no disturbance. This reduced uncharacteristic mixing due to rapid movements of stressed fish. To minimise this acclimatization period the same fish were used wherever possible throughout a day. One run for each treatment was carried out.

5.4.5 Calculations and statistical analyses

Time corrections

The time taken for the tracer to travel from the tank outlet sample point to the spectrophotometer cell was finite. To ensure accurate response times, measured from the time of first injection, a correction as calculated below was applied.

Water exited the treatment tank through 10 holes in the outlet collection pipe. The location of these holes ranged from proximal, closest to the external standpipe and sample point, to distal holes located at the extreme ends of the collection bar closest to the tank walls. The time for water to travel between the inside of the tank and the spectrophotometer cell was therefore partly related to the point of entry into the collection pipe. Tracer injected at the tank outlet pipe was used to estimate the time taken for water to travel from the tank to the spectrophotometer. 5 replicate time measurements from both the proximal and distal sections of the collection pipe were conducted, with the peristaltic pump

set at a constant 47 ml min^{-1}. Results, to be used in further calculations are shown in Table 5.5.

Table 5.5: Time taken for water to travel from the treatment tank to the spectrophotometer cell

Time (s)	
Proximal	Distal
12	15
12	15
11	16
12	13
11	15
11.6	14.8
mean 13.2	

The speed of the trace of absorbance against time on the chart paper was known, so a distance equivalent to 1 min 13 s was marked back from the point which noted 1 min from the start of the experiment. A corrected start time of experiment, or time of first tracer injection was obtained. All times were measured from this corrected time.

Quantification of mixing

The following values were measured: time to first response; time to maximum tracer concentration; maximum tracer concentration; and the tracer concentration every 2 min from the start of the experiment. The appropriate calibration curve (Figure 5.3) was used in the conversion

of absorbance values to tracer concentration, measured as ml tracer l water^{-1}.

Exit age distribution curves (E-curves) were constructed from the tracer concentration results measured at 2 min intervals, as described in section 5.3.2, from Levenspiel (1966). The following five parameters quantifying the E-curves and therefore the mixing in the treatment tanks were calculated using methods described mathematically by Cholette and Cloutier (1959) and more practically by Klapsis (1983) and Burley (pers. comm., 1989).

Calculated mean residence time (t_c) is the actual residence time which occurred during each treatment (see section 5.3.2), calculated as:

$$t_c = \frac{\text{Sum of concentrations * time}}{\text{Sum of the concentrations}} = \frac{\hat{E}(Ct)}{\hat{E}C} \text{ (min)}$$

Ideal mean residence time (t_i) is the theoretical residence time, which is seldom achieved in practice, calculated as:

$$t_i = \frac{\text{Working volume of the tank (l)}}{\text{Volume flow into the tank (l min.}^{-1}\text{)}} = \frac{V}{Q} \text{ (min)}$$

The ratio t_c:t_i a measure of mixing, used by Burrows and Chenoweth (1955) and Burley and Klapsis (1985) to compare tanks of different treatments or even designs, is calculated as:

$$\theta_c = \frac{\text{Calculated mean residence time}}{\text{Ideal mean residence time}} = \frac{t_c}{t_i}$$

Higher values of $t_c:t_i$ indicate a greater degree of mixing, which for the purposes of this study can be considered as beneficial.

Dead volume (V_d) is an estimate of the volume of the regions in a tank which are by-passed by the main flow, calculated as:

$$V_d = V(1-\theta_c) \quad (1)$$

Where: V = Tank working volume (l)

Percentage dead volume ($\%V_d$) is an estimate of dead volume which is comparable between tanks containing different volumes of water. Use of this measure in previous studies cannot be found.

$$\%V_d = \frac{V_d}{V} \times 100$$

Obviously in aquaculture tanks it is desirable to reduce V_d and $\%V_d$ as much as possible. An exception to this policy is the production of still regions within a tank to settle out suspended solids, but this was not required in this study. In practice it is obviously not possible to achieve negative V_d and $\%V_d$. Theoretically however V_d and $\%V_d$ will be negative if t_c is greater than t_i. Results which indicated such negative values were retained for statistical purposes so that trends in the

data could be indicated and regression lines for predictive purposes could be drawn. The reasons for such results will be discussed in section 5.6.3.

The standard deviation (s), a measure of the spread of the residence times about the mean of the E-curve was calculated as:

$$s = \sqrt{E(c-c_m)^2 / n-1}$$

A dimensionless value of standard deviation (s_d) used in this study was calculated as:

$$s_d = \frac{s}{t_c}$$

It was considered preferable to achieve as low a value of s_d as possible, indicating a narrow spread of residence times and therefore a more plug flow situation.

Mixing parameter data, calculated on an IBM compatible PC system using "VP Planner" spreadsheets, were transferred via ASCII print files to a "Minitab" statistics programme on a DEC VAXcluster mainframe computer system, for statistical analysis.

Analysis of results

Tank mixing data was analysed to investigate both the influence of various treatments on mixing and to correlate any significant or obvious influences with data from other

sections of this study, eg. fish growth and water quality aspects. A five stage method of hydrodynamic data analysis was adopted as standard unless otherwise stated.

1. Plots were constructed illustrating the distribution of each hydrodynamic parameter with respect to each treatment parameter, eg. water depth and flow rate (eg. listed in section 5.5 as "water depth vs flow rate"), to examine the magnitude and nature of any associations between the two sets of data and to assess the most appropriate means of statistical evaluation. For reasons explained above, triplicate freshwater results were used.
2. The correlation coefficient for each plot was calculated to measure the extent of any linear correlation.

3a. Non significant correlations were either described non-statistically or the data were transformed and a correlation coefficient recalculated.

3b. The regression equation describing the association between significantly correlated data was calculated.

4. Stages 1 - 3 were repeated using saltwater data
5. Fresh and saltwater distributions were compared statistically and any significant differences were noted. The method of comparison was dependant on the nature of the distribution. Either the gradients of the regression lines, or the mean or median of the two distributions, were compared. The level of significance of any differences could not be

determined with a two-way analysis of variance because the number of fish and no-fish replicate results were not balanced.

Five hydrodynamic mixing parameters (section 5.4.5) were calculated as indicators of fluid mixing in the treatment tanks : first response time, the ratio t_c/t_i, dead volume, % dead volume and dimensionless standard deviation.

Unless otherwise stated, the term "residence time" refers to ideal mean residence time (t_i).

Four series of experiments were conducted to examine the effect on tank hydrodynamics of: depth at a constant flow rate; depth at a constant residence time; flow rate; and stocking density of turbot.

1. Comparisons between each hydraulic mixing parameter and the depth of water in the treatment tanks, at a constant flow rate and variable residence times, were plotted. The correlation coefficient was calculated for each comparison. The depth of water in the treatment tanks was within the range 6 - 18 cm. Corresponding residence times were 6.18 - 18.54 min unless otherwise stated. Flow rate was maintained at a constant 2 l min^{-1}. For comparison, replicate treatments were carried out with fish present in the tanks at the stocking densities used in the growth experiments. Treatment tanks which

contained constant stocking densities of fish, calculated with respect to both surface area (SD_a) and volume (SD_v) were analysed.

2. Comparisons between each hydraulic mixing parameter and the depth of water in the treatment tanks, in which there was a constant residence time and variable flow rates, were plotted. The correlation coefficient was calculated for each comparison. Results refer to depths of water in the treatment tanks within the range 6-18 cm. Corresponding flow rates were 0.57 - 3.43 l min^{-1} unless otherwise stated. Ideal mean residence time was maintained at a constant 10.8 min. Replicate treatment tanks containing stocking densities of fish, with respect to both surface area (SD_a) and volume (SD_v) were analysed.

3. Comparisons between each hydraulic mixing parameter and the flow rate of water into the treatment tanks, with a constant depth of 9 cm and volume of 18.54 l, were plotted. The correlation coefficient was also calculated for each comparison. Results refer to flow rates within the range 0.5 - 13 l min^{-1} unless otherwise stated. Replicates of each flow rate treatment were carried out with 10 fish $tank^{-1}$ present as per a growth experiment. Data for which a non-linear association was apparent, were transformed to produce a linear regression line of best fit. The transformation which produced the most significant regression line, determined by analysis of variance about the line, was adopted.

4. Comparisons between each hydraulic mixing parameter and the stocking density of turbot in the culture tanks were plotted. Depth (9 cm), volume (18.54 l), ideal mean residence time (9.27 min) and flow rate (2 l min^{-1}) were constant at each stocking density treatment. The correlation coefficient was calculated for each comparison shown, though only quoted where significant. Fish were taken randomly from a population with a size range of 2.8 - 14.9 g (Tables 5.2 and 5.3), so stocking density was measured as number of fish $tank^{-1}$. Results refer to stocking densities within the range 0 - 50 fish $tank^{-1}$, equivalent to a mean of 0 - 1.84 kg m^{-2}, unless otherwise stated.

5.5 RESULTS

5.5.1 Depth - constant flow rate

No skews in the frequency distribution of any of the hydrodynamic mixing parameters were evident although the number of replicates was insufficient for normality to be formally tested reliably. It was therefore considered reasonable to apply normal statistics with care, but to use non-parametric tests in cases of doubt.

Unless otherwise stated results from tanks with and without fish were not significantly different.

First response time vs. depth (Figure 5.7): first response time increased marginally with increased depth between 9 and 18 cm. This increase was not significant because of the large within-depth variation. At the shallowest depth tested (6 cm) first response time was highest at over 1 min, approximately double that of the other treatment tanks.

t_c/t_i vs. depth (Figure 5.8): overall, t_c/t_i appeared unaffected by depth. Within a depth range of 9 - 18 cm however, t_c/t_i decreased significantly with increased depth ($y=1.04-0.0131x$, $r=-0.700$, $df=10$, $p<0.05$) from a mean of 0.937 at 9 cm to 0.848 at 18 cm.

V_d vs. depth (Figure 5.9): V_d and depth were significantly correlated ($r=0.779$, $df=13$, $p<0.001$). V_d increased with increased depth, greater than 9 cm. A regression equation was not calculated for the data within the range 6 - 18 cm, because a clear non-linear association was evident. Between 9 and 18 cm depth, V_d increased significantly with increased depth ($y=-4.21+0.62x$, $r=0.844$, $df=10$, $p<0.001$). Extrapolation of this regression line indicated that dead volume may be eliminated at depths less than 6.79 cm. An approximate curve of best fit which included data from the 6 cm depth tank indicated that a minimum dead volume of only 0.7 l at 7.9 cm may however have been achieved. The V_d in a 6 cm deep tank did not conform to the overall trend. A similar though not significantly correlated distribution occurred

Figure 5.7: The influence of water depth on the first response time in tanks with the same flow rate (2 l/min)

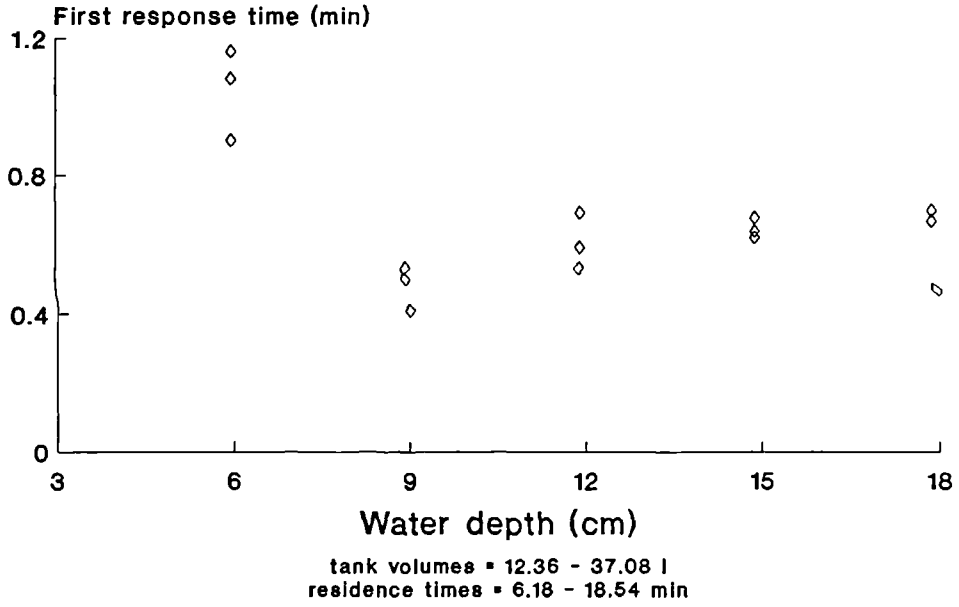
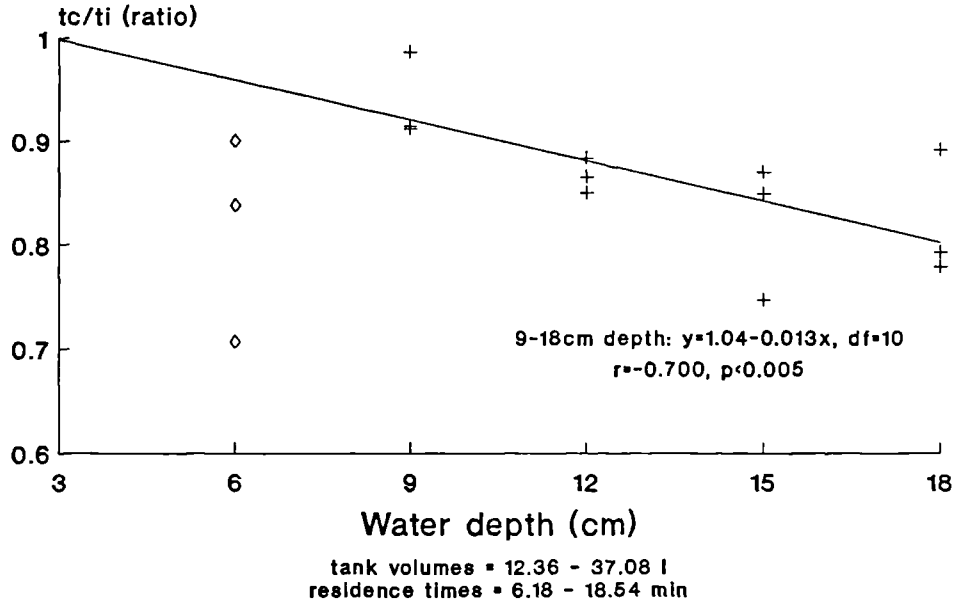


Figure 5.8 The influence of water depth on tc/ti as an indication of mixing in tanks with the same flow rate (2 l/min)



with fish present in the treatment tanks.

$\%V_d$ vs. depth (Figure 5.10): $\%V_d$ was not significantly correlated with depth between the 6 and 18 cm depth range tested, but a significant correlation resulted ($y=-3.79+1.31x$, $r=0.700$, $df=10$, $p<0.05$) within the range 9 to 18 cm depth. Extrapolation of this regression line indicated that $\%V_d$ may be reduced to 0 at depths below 2.89 cm. Alternatively an approximate, curve of best fit which described the association and including data from the 6 cm deep tank, indicated that $\%V_d$ approached a minimum of 6.7 % at a depth of 9 cm. This latter, approximate estimation of minimum dead volume is similar to the approximate estimate calculated from V_d data, but regression lines for the two sets of data, excluding the 6 cm depth tank, differ by 3.9 cm depth in the estimate of the water depth required to eradicate dead volume.

s_d vs. depth (Figure 5.11): s_d decreased significantly with increased depth ($r=-0.792$, $df=13$, $p<0.001$), but the relationship did not appear strictly linear with s_d values corresponding to 6 cm depth included. s_d also decreased significantly with increased depth between 9 and 18 cm ($y=4.56\times10^{-3}-1.95\times10^{-4}x$, $r=-0.981$, $df=10$, $p<0.001$). At shallow depths a greater quantity of water was therefore taking longer to pass through the tanks than the ideal mean residence time, indicating that mixing was less stable at shallow depths.

Figure 5.9 The influence of water depth on dead volume in tanks with the same flow rate (2 l/min)

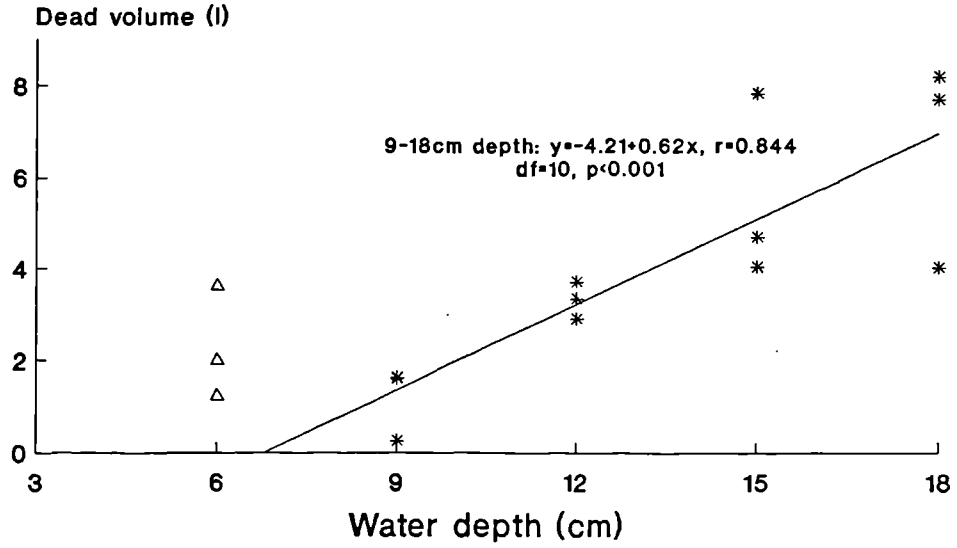
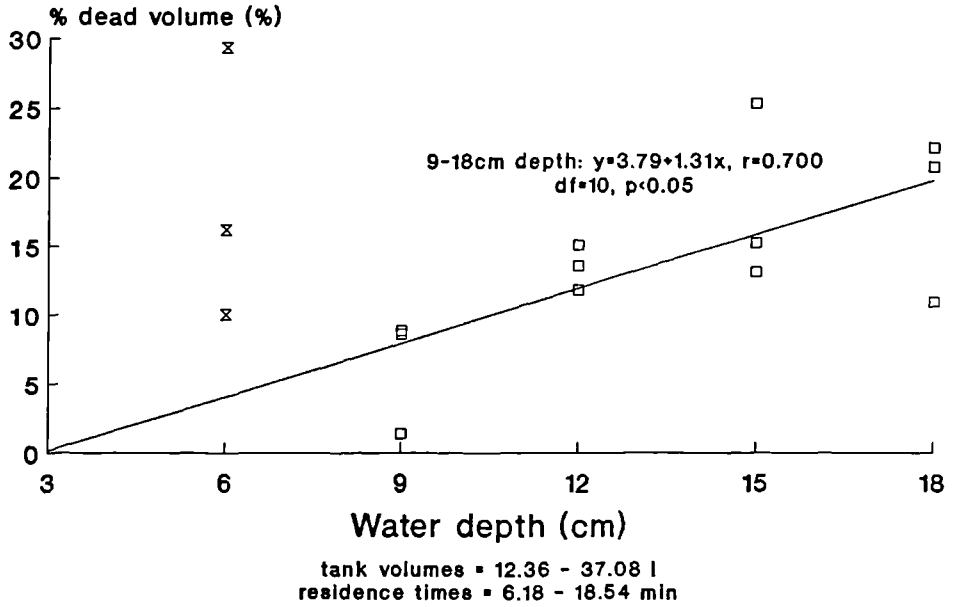


Figure 5.10 The influence of water depth on percentage dead volume in tanks with the same flow rate (2 l/min)



Summary: within the depth range 9 - 18 cm, t_c/t_i and sd decreased while V_d and $\%V_d$ increased with increased depth. These parameters were all measures of mixing, so mixing clearly improved (ie. approached perfect mixing) with decreased depth at a constant flow rate.

5.5.2 Depth - constant residence time

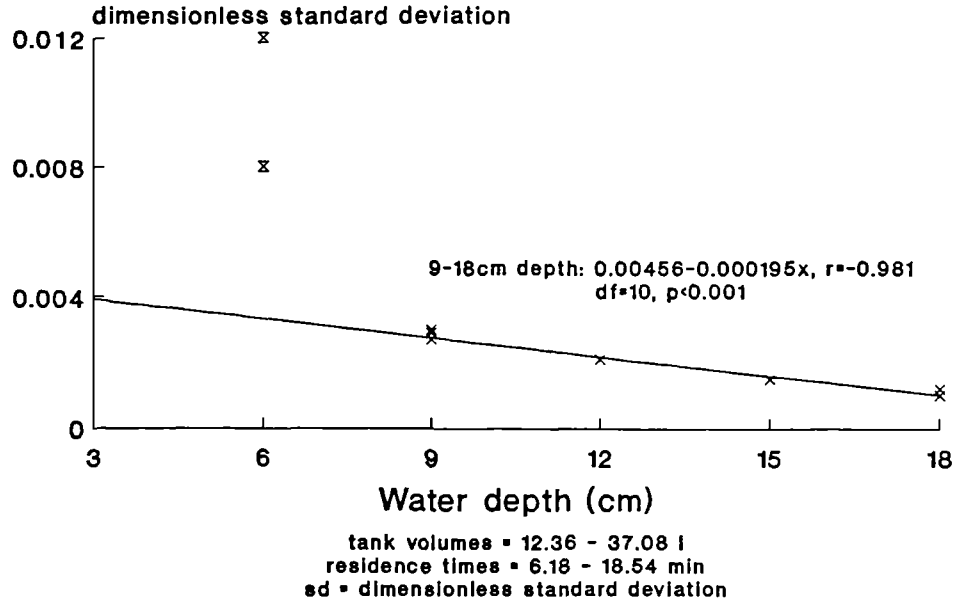
Unless otherwise stated results from treatments tanks with and without fish were not significantly different.

First response time vs depth (Figure 5.12): first response time was not significantly influenced by depth between 9 and 18 cm, but was appreciably longer at 6 cm depth. Values showed a similar distribution and magnitude to those described in Figure 5.7 for which flow rate was constant and residence time was varied.

t_c/t_i vs. depth (Figure 5.13): triplicate mean t_c/ti ratios increased from 0.883 at 6 cm and 0.908 at 18 cm, to a peak of 1.142 at 15 cm depth. It appeared that, between 3 - 15 cm depth, mixing increased with increased depth, contrary to that indicated in Figure 5.8. No trends in the change of t_c/t_i with respect to depth, were observed with fish present in the treatment tanks.

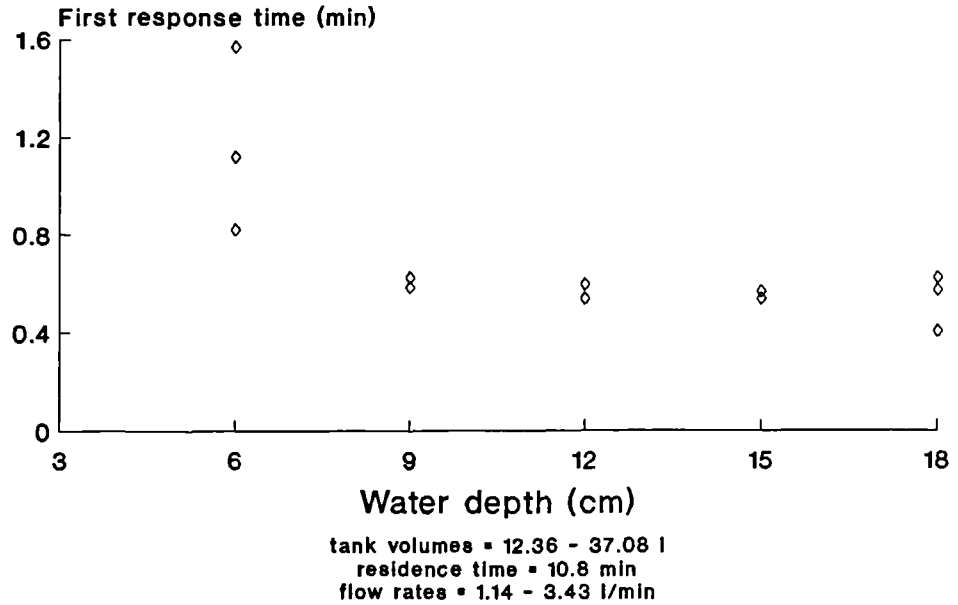
V_d and $\%V_d$ vs. depth (Figures 5.14 and 5.15): both V_d and $\%V_d$ followed a similar distribution with respect to depth. Minimum dead volumes occurred in tanks with a

Figure 5.11 The influence of water depth on sd of the E curve distributions in tanks with the same flow rate (2 l/min)



tank volumes = 12.36 - 37.08 l
residence times = 6.18 - 18.54 min
sd = dimensionless standard deviation

Figure 5.12: The influence of water depth on first response time in tanks with the same residence time



tank volumes = 12.36 - 37.08 l
residence time = 10.8 min
flow rates = 1.14 - 3.43 l/min

Figure 5.13: The influence of water depth on tc/ti in tanks with the same residence time

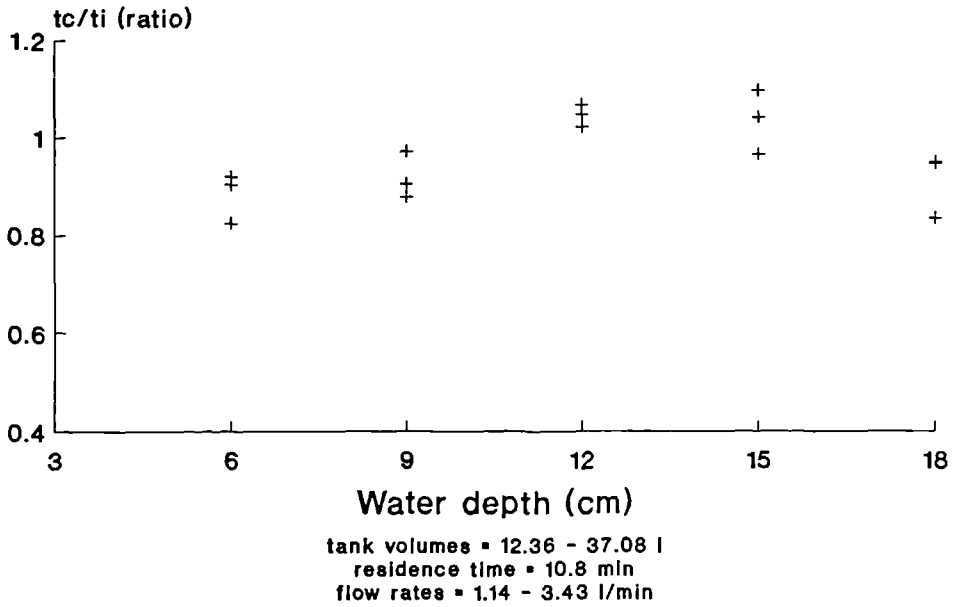
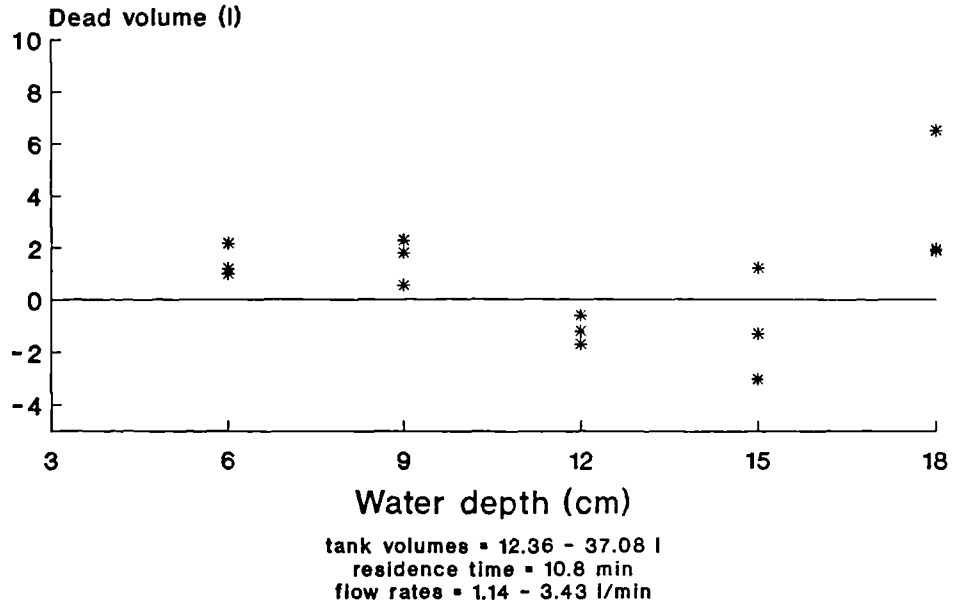


Figure 5.14: The influence of water depth on dead volume in tanks with the same residence time



water depth of 15 cm (-0.44 l, -14.23%), though this trend was not definite. There were no significant associations between dead volume and depth, so estimates of the depth at which dead volume would have been eliminated could not be made. No trends in the change of V_d and $\%V_d$ were observed with fish present in the treatment tanks.

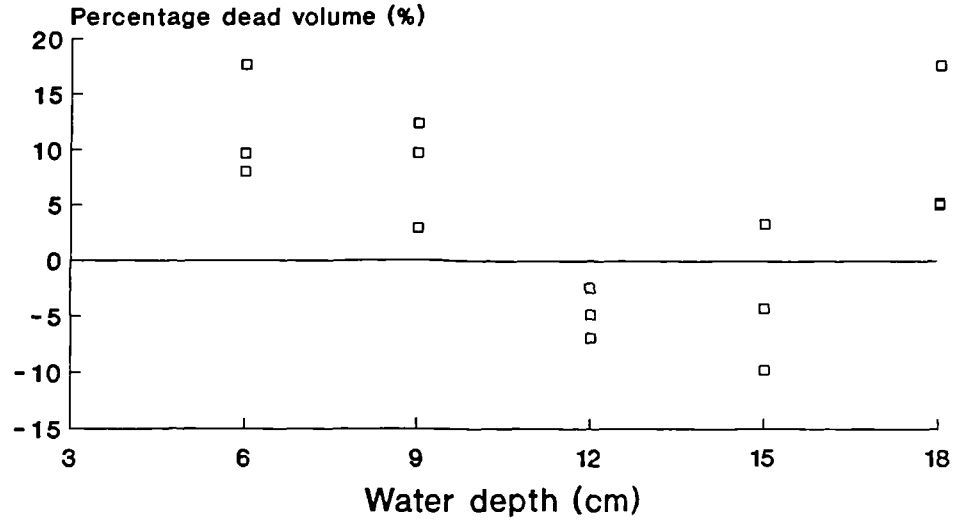
s_d vs. depth (Figure 5.16): s_d decreased significantly with increased depth ($y=6.80x10^{-3}-3.37x10^{-4}x$, $r=-0.881$, $df=13$, $p<0.001$). Similar distributions occurred with fish present at a constant SD_a ($y=6.4x10^{-3}-3.07x10^{-4}x$, $r=-0.934$, $df=3$, $p<0.05$) and a constant SD_v ($y=5.2x10^{-3}-2.21x10^{-4}$, $r=-0.984$, $df=3$, $p<0.01$). These three regression equations and the equation for the corresponding regression of s_d and depth at a constant flow rate, described similar lines. The extent of mixing and hold-back within the treatment tanks, as indicated by the spread of the residence times of the E distribution curve, increased with increased depth as expected.

Summary: only s_d was significantly influenced by depth at a constant residence time. sd decreased with increased depth. t_c/t_i appeared to increase while Vd and %Vd appeared to decrease with increased depth to 15 cm, indicating the mixing was possibly maximal at this depth.

5.5.3 Flow rate

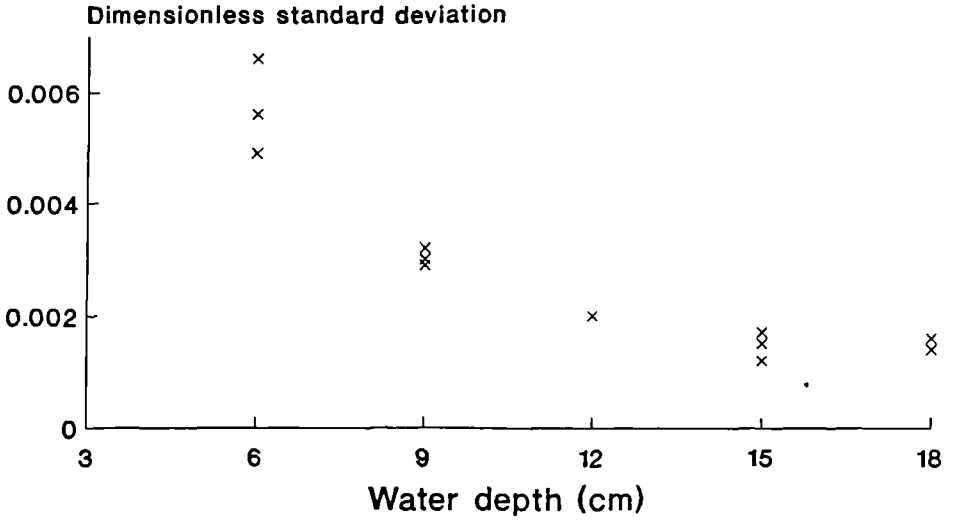
First response time vs flow rate (Figure 5.17): first

Figure 5.15: The influence of water depth on percentage dead volume in tanks with the same residence time



tank volumes = 12.36 - 37.08 l
residence time = 10.8 min
flow rates = 1.14 - 3.43 l/min

Figure 5.16: The influence of water depth on the sd of the E distributions in tanks with the same residence time

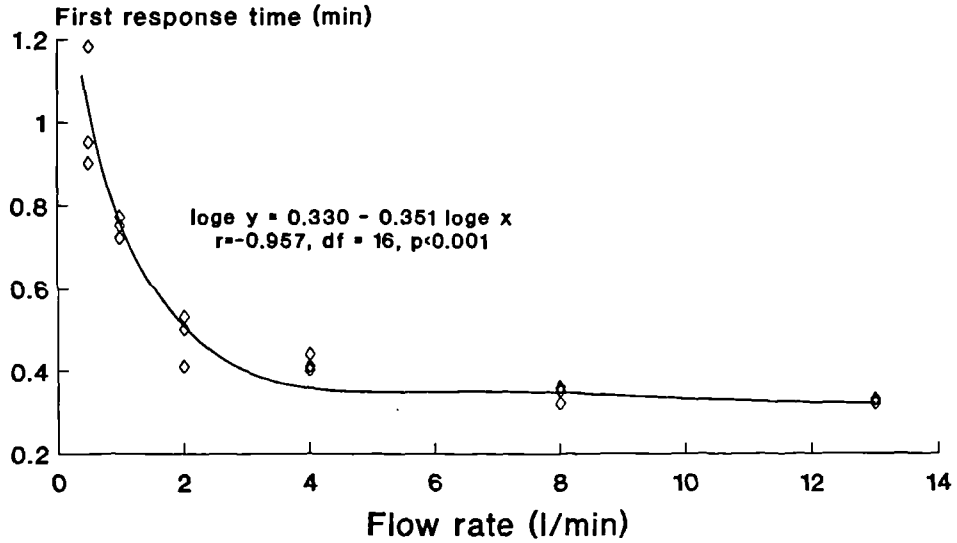


tank volumes = 12.36 - 37.08 l
residence time = 10.8 min
flow rates = 1.14 - 3.43 l/min

response time decreased significantly with increased flow rate ($r=-0.727$, $df=16$ $p<0.001$). A reduction in the decrease of first response time with increased flow was evident. On average an increase in flow rate from 0.5 to 13 l min^{-1} produced a 68 % decrease in first response time from 1.01 to 0.33 min. The association was clearly not linear. An increased correlation coefficient of $r=-0.958$ ($df=16$, $p< 0.001$), after a logarithmic transformation of both variables, confirmed this suggestion. The significant association was described by the equation $\log_e y=0.330-0.351 \log_e x$. A replicate treatment with a stocking density of 10 fish $tank^{-1}$, produced a similar regression equation ($\log_e y=0.218-0.434\log_e x$). The time taken for the tracer to travel from the inlet to the outlet of the treatment tanks decreased with increased flow rate, as a result of either quicker mixing or increased short circuiting. Figure 5.17 indicates that first response time was greatly reduced by a small increase in flow rate below approximately 2.5 l min^{-1}. At greater values, little change in first response time was achieved despite large changes in flow rate, relative to the scale of flow rates studied.

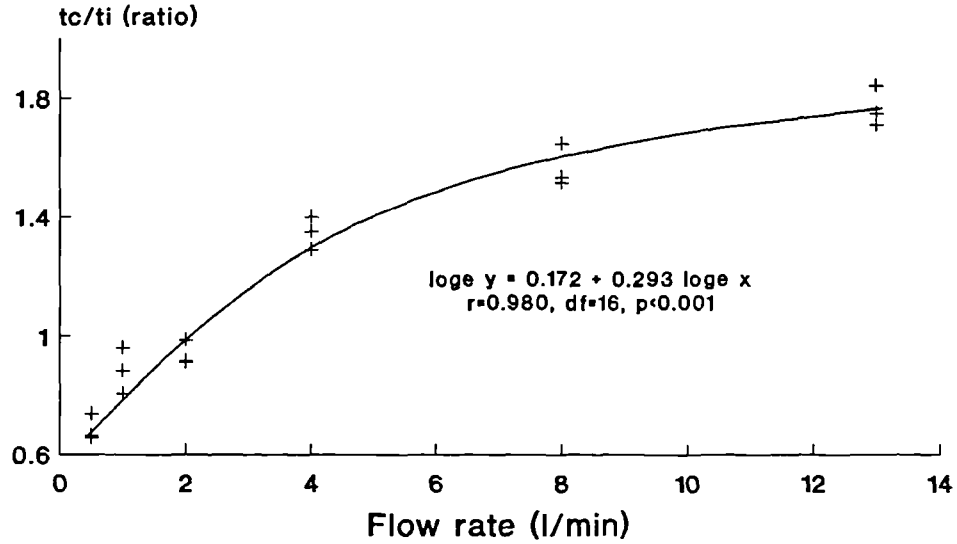
t_c/t_i vs flow rate (Figure 5.18): while t_c/t_i increased significantly with increased flow rate ($r=0.939$, $df=16$, $p<0.001$) the association was not linear and was best described by the transformed regression equation $\log_e y=0.172+0.293\log_e x$ ($r=0.980$, $df=16$, $p<0.001$). The rate of increase of t_c/t_i decreased with increased flow

Figure 5.17: The influence of flow rate on the first response time of dye passing through tanks



tank volume = 18.54 l, water depth = 9cm
residence times = 1.43 - 37.08 min

Figure 5.18: The influence of flow rate on tc/ti as a measure of mixing in the treatment tanks



tank volume = 18.54 l, water depth = 9cm
residence times = 1.43 - 37.08 min

rate from a mean of 0.685 at 0.5 l min^{-1} to 1.763 at 13 l min^{-1}. Extrapolation of the curve produced a maximum t_c/t_i value of approximately 1.8 at 18 l min^{-1}. At flow rates greater than 18 l min^{-1} t_c/t_i was not expected to increase. Replicate treatments with fish present in the treatment tanks produced a similar regression equation ($\log_e y=0.880+0.274\log_e x$, $p<0.01$).

V_d $\%V_d$ vs. flow rate (Figure 5.19): the distribution of both parameters was the same, with differences due only to scaling, because the tank volume was maintained constant at 18.54 l. V_d and $\%V_d$ decreased significantly with increased flow rate ($r=-0.939$, $df=16$, $p<0.001$), and dead volume was eradicated at flow rates greater than 2.37 l min^{-1}. Logarithmic transformation of flow rate data produced an increased correlation ($r=-0.975$, $df=16$, $p<0.001$). The equations describing the association between V_d and $\%V_d$, and $\log_e$ flow rate were $y=2.63-6.23\log_e x$ and $y=14.2-33.6x$ respectively, and with fish present: $y=2.22-5.08\log_e x$, ($p<0.01$) and $y=12.0-27.4\log_e x$ ($p<0.01$) respectively. Examination of the former two regression equations indicated that dead volume may have been eradicated at flow rates in excess of 2.64 l min^{-1}.

s_d vs. flow rate (Figure 5.20): s_d, a measure of the spread of the residence times, increased significantly with increased flow rate ($r=0.476$, $df=16$, $p<0.05$). Examination of Figure 5.20 indicated that the rate of increase of s_d appeared to be reduced with increased flow

Figure 5.19: The influence of flow rate on the dead volume of water in the treatment tanks

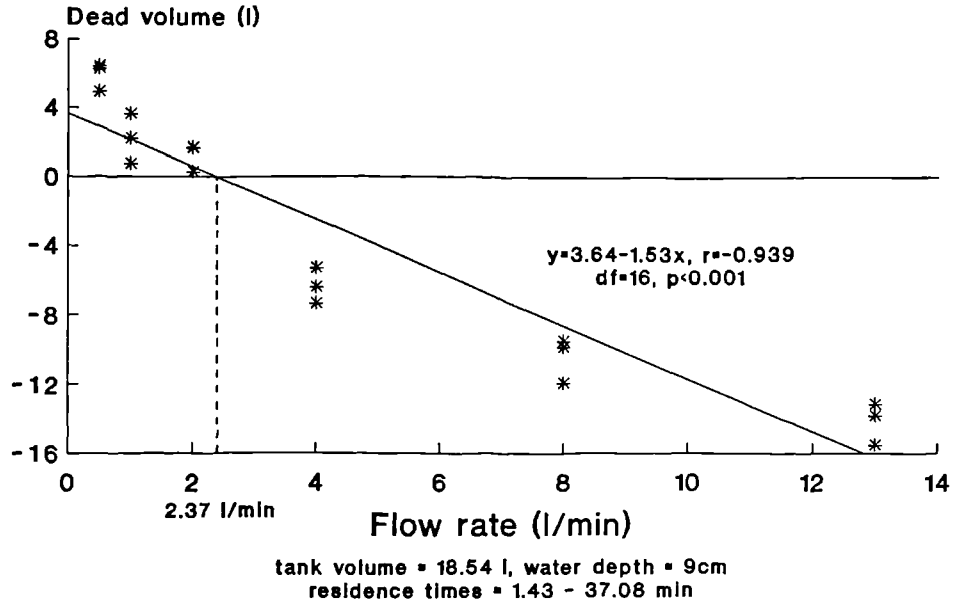
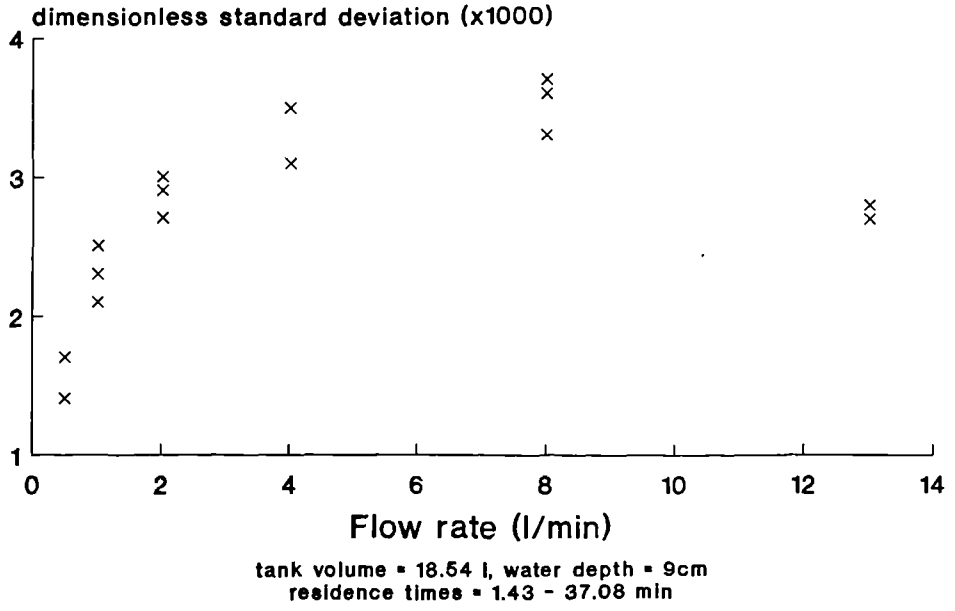


Figure 5.20: The influence of flow rate on the sd of the distribution of the E curves for the treatment tanks



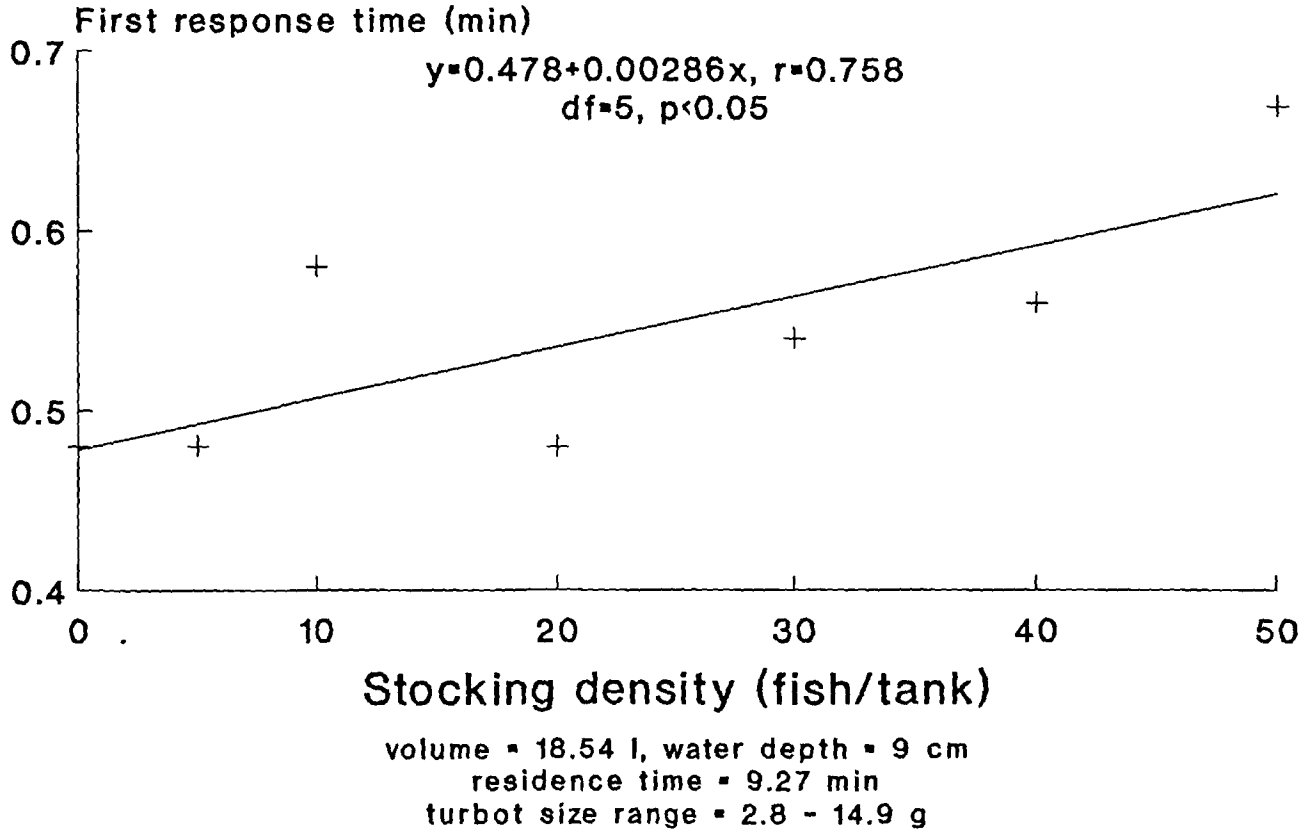
rate between 0.5 and 8 l min^{-1}, and that there was a reduction in s_d at 13 l min^{-1}. s_d was plotted against log_e of flow rate to produce a greater correlation ($y=2.29x10^{-3}+4.52x10^{-4}log_e x$, $r=0.776$, $df=16$, $p<0.001$). Data from replicate treatment tanks with fish present produced a regression line of $y=2.48x10^{-3}+8.52x10^{-4}log_e x$.

Summary: the log_e of first response time decreased significantly with increased log_e of flow rate; log_e t_c/t_i increased significantly with increased log_e of flow rate; log_e V_d and log_e $\%V_d$ decreased significantly with increased log_e of flow rate; and s_d increased significantly with increased log_e of flow rate. Mixing therefore increased with increased flow rate. Changes in flow rate at flows greater than approximately 2.5 l min^{-1} produced only small changes in first response time. A calculated minimum flow rate of 2.64 l min^{-1} was estimated to eradicate V_d in the treatment tanks under the conditions specified.

5.5.4 Stocking density

First response vs. stocking density (Figure 5.21): first response time increased significantly with increased stocking density ($y=0.478+2.86x10^{-3}x$, $r=0.758$, $df=5$, $p<0.05$). Figure 5.21 shows an acceleration in the increase of first response time with increased stocking density throughout the stocking density range tested. It was expected that first response time would decrease with

Figure 5.21: The influence of stocking density of turbot on the first response time of dye passing through the tanks



increased stocking density as the movement of the fish would increase mixing. This situation was not however observed.

t_c/t_i vs. stocking density: no significant trend was recognised. t_c/t_i values were distributed within the range 0.898 - 0.982.

V_d vs. stocking density and $\%V_d$ vs. stocking density: no significant association was recognised. V_d and $\%V_d$ values were distributed within the range 0.33 l, 1.8 % to 1.89 l, 10.2 % respectively.

s_d vs. stocking density: also no significant correlation was determined. s_d values varied between 2.9×10^{-3} and 3.5×10^{-3}.

Summary: the presence of fish, at the stocking densities used in this experiment did not significantly influence mixing in the treatment tanks, other than an increase in first response time with increased stocking density.

5.6 DISCUSSION

5.6.1 Depth - constant flow rate

Mixing was shown to significantly improve, with decreased water depth in the treatment tanks, as indicated

by increased t_c/t_i and decreased V_d and $\%V_d$, at a constant flow rate. Three explanations may account for this correlation. Firstly, changes in residence time (t_i) rather than depth may have caused the association. Residence time increased as a factor of increased depth, therefore mixing was also correlated with residence time. For this reason an experiment to indicate mixing at a constant residence time was also conducted, replicating the depth range. No correlation between mixing and depth was then determined.

Secondly, the association may have indicated an influence of depth itself. This was expected, because less water volume was available in shallower tanks for eddies and dead areas to exist in regions which were not in the path of the main flow, between tank inlet and outlet. Effectively as depth was decreased the flows would be forced, by the decrease in cross-sectional area of the fluid, to become more laminar. A more plug-flow, rather than perfectly mixed, situation would be approached, hence the improvements in the hydrodynamic parameters which occurred.

Thirdly the constant flow rate was dissipated into a greater volume of water as depth was increased, as was found by Burley and Klapsis (1985), so eddies and flow separations were more likely to occur. The stability of the flows decreased with increased depth though the reverse situation was expected had depth itself been

primarily responsible for mixing.

The lack of significant correlations between depth and mixing at a constant residence time indicates that the first explanation, of the correlation between depth and mixing at a constant flow rate, is the most likely, as discussed in the following section.

Between 9 and 18 cm depth, the influence of depth at a constant flow rate, on the tank hydrodynamics was linear and so could be defined using linear regression equations. These indicated the maximum depth at which dead volumes would be expected to be absent, provided that flow rate was not altered. This maximum depth value indicates at which depth the water was used most efficiently. Assuming a constant flow rate, at greater depths dead volumes will be present and therefore water will not be used efficiently, while at shallower depths no further improvement in reduction of dead volumes is achieved and other factors such as fish stress may become more important. Estimates of the depth at which dead volume would have been eliminated, or reduced to a minimum, were variable, depending on the method of calculation, ranging from 0 % at 2.89 cm to 6.7 % at 9 cm, calculated from $\%V_d$ data. Estimates using V_d data were more consistent, resulting in a total elimination of dead volume at a depth of approximately 7 cm. While the variation in optimal depth was evident, all the estimates were at the lower end of the depth range studied. This indicates that the

treatment tanks were better mixed when only partially filled, rather than when filled to capacity.

Hydrodynamic data from the tank filled to 6 cm did not conform to the data from tanks with a depth between 9 and 18 cm. Clearly at this depth the association between depth and mixing parameters was different to that at greater depths. This may have been due to: differences in flow caused by the design of the treatment tank, eg. height of the inlet pipe; or to inherent hydrodynamic differences, eg. skin friction. The former explanation is thought to be more likely. The inlet spray pipe jets were located 7 cm above the bottom of the tank and therefore 1 cm above the water level in the 6 cm deep tank. Water entering a tank containing 6 cm of water did so vertically, so the momentum of the water was dissipated by the tank floor. Water entering the tank with a greater water depth than 7 cm did so horizontally, so the momentum of the inflow was dissipated horizontally, towards the outlet. Clearly these are two very different mixing situations, hence the difference in mixing results. Under such circumstances the exclusion of the 6 cm depth results from many of the calculations appears justified. It should also be noted that because the results indicated such an abnormality, the method of analysis of mixing is verified. Further, as mixing was shown to be worse in the tank with the inlet pipe above the water, tanks used in this study should be maintained with a depth greater than 7 cm. The outlet from the tanks was located as near to

the tank floor as possible to facilitate the removal of wastes. To reduce short-circuiting of water from the inlet to the outlet, the inlet was located at a different height above the tank floor than the outlet. The height of the inlet was then a compromise between the optimum height at each water depth tested. Experiments conducted after construction indicated that the height adopted was not suitable for the shallowest tanks. It should be noted that at flow rates greater than 2 l min^{-1} the inlet jet stream which was not immersed would become more horizontal.

5.6.2 Depth - constant residence time

Mixing in the treatment tanks was not significantly affected by alterations in depth, providing the residence time was constant. The standard deviation of the distribution of residence times, shown by the E-distribution curves, decreased with increased depth, indicating that flows were less stable at shallower depths. This was opposite to the situation in which the flow rates were constant.

A comparison between t_c/ti, V_d and $\%V_d$ results from tanks with a constant flow rate and tanks with a constant residence time indicated that alteration of flow rate at a constant residence time, to produce the depth range 9-18 cm, did little to influence these hydrodynamic parameters. Alteration of residence time at a constant

flow rate, to produce the same depth range, did however significantly affect the parameters. This indicated that it was the adjustment of residence time rather than depth itself which influenced mixing in the particular treatment tanks studied, all other conditions being equal.

A step-wise, simplified, generalization indicates the experimental and conclusive rationale for this conclusion:

1a. Changes in depth at a constant flow influenced mixing.

1b. Changes in residence time at a constant flow influenced mixing.

2. Does depth or residence time therefore cause this influence?

3. To determine this, residence time was kept constant, and depth was altered at varying flow rates.

4a. Changes in depth at a constant residence time did not influence mixing.

4b. Changes in flow rate at a constant residence time did not influence mixing.

5. Therefore mixing was influenced primarily by residence time rather than depth or flow rate.

The mean V_d, within the range 9 - 18 cm depth at varying flow rates and a constant residence time of 10.8 min was 0.62 l. The V_d, calculated from the regression equation describing the association between V_d and the same range of depths, at a constant flow rate and variable

residence time, was 2.49 l at a residence time of 10.8 min. These two V_d values are not equal, so residence time was unlikely to be the sole determinant of V_d.

5.6.3 Flow rate

The reduction in first response time concurrent with increased t_c/ti and associated decreases in V_d and $\%V_d$ as flow rate was increased, indicated that the reduction in first response time was due primarily to improved (ie. increased) mixing in the treatment tanks rather than purely an increase in short-circuiting. Increases in flow rate up to approximately 2.5 l min^{-1} decreased first response time markedly. Dead volumes were eradicated at flow rates above 2.64 l min^{-1} and mixing, as measured by t_c/t_i, increased with increased flow rates up to 18 l min^{-1}. A flow rate of at least 2.64 l min^{-1} was therefore sufficient to produce mixing with no dead volumes within the treatment tank. Flow rates below this value produced inadequate mixing as indicated by reduced first response times (proved in this instance to be primarily a measure of mixing rather than short-circuiting), low t_c/t_i values and the presence of dead volumes. Flow rates above this value increased mixing, but with negligible hydrodynamic benefits. The stability of mixing decreased with increased flow rate as mixing became more turbulent.

The depth, and therefore volume in the tank was

constant throughout the flow rate experiments. Volume is equivalent to the product of flow rate and residence time, so residence time varied as a factor of flow rate. Mixing parameters therefore correlated with residence time to the same level of significance as with flow rate.

It has been stated in section 5.4.5 that in practice it is not possible to achieve negative V_ds. Theoretically however V_d and $\%V_d$ will be negative if t_c is greater than t_i. In this study, this was found to occur at flow rates of 4 l min^{-1} and greater. The mixing model adopted in this study assumed that a proportion of the main flow, from the inlet direct to the outlet, was recycled within the tank to form a region of poor water exchange, ie. a dead volume. At actual flow rates of 4 l min^{-1}, or estimated flow rates of greater than 2.37 l min^{-1} a different mixing situation occurred. The tank was well mixed throughout, with no V_d, so the proposed model was not appropriate and the calculation of V_d and $\%V_d$ was not applicable. Negative V_d and $\%V_d$ values were however calculated because these facilitated the quantification of the change in V_d with various parameters, using regression analyses.

5.6.4 Stocking density

With the exception of first response time, results indicated that, within the range of stocking densities used in this experiment, the presence of fish did not

significantly influence the hydrodynamics of the treatment tanks. While the investigation into the effect of stocking density on mixing was conducted under only one treatment regime, the results obtained, corroborated by the comparisons between the results from treatment tanks which did and did not contain fish, made throughout the study, suggest that the triplicate mean results obtained from treatment tanks containing no fish, were representative of the single results obtained from tanks containing fish. The hydrodynamics of the tanks with no fish were therefore compared with other non-physical parameters such as growth rate and water quality in tanks with the same treatments.

5.6.5 Comparisons with other studies

Little comparable data has been published regarding the quantification of mixing in aquaculture tanks. Burrows and Chenoweth (1955) compared mixing in three types of ponds and produced "effective detention times", equivalent to t_c/t_i values. Curiously, in a follow-up study Burrows and Chenoweth (1970) did not quantify mixing in this way. Burley and Klapsis (1985) quoted several t_c/t_i values in a study of adaptions to, and stocking density effects in, round-cornered 1 m square tanks. These are summarised in Table 5.6, together with representative results from the present study.

Table 5.6 Comparison between published mixing study results

ref	tank	treatment	t_c/t_i	length (m)	width (m)	depth (m)	flow (l min^{-1})
1	Foster Lucas		0.83	23.18	5.19	0.915	919.1
1	Circular		0.88	7.62	-	0.769	477.8
1	Rect raceway		1.11	24.40	2.44	0.610	2229.5
2	Square		0.75	1.00	1.00	0.25	10
2	Square		1.13	1.00	1.00	0.10	8
2	Square		0.99	1.00	1.00	0.10	10
2	Square	*	1.20	1.00	1.00	0.10	10
3	Rect.	const flow	0.94	0.565	0.355	0.09	2
3	Rect.	const flow	0.85	0.565	0.355	0.18	2
3	Rect.	const t_i	0.88	0.565	0.355	0.06	1.14
3	Rect.	const t_i	1.14	0.565	0.355	0.15	2.86
3	Rect.	variable flow	0.69	0.565	0.355	0.09	0.5
3	Rect.	variable flow	1.77	0.565	0.355	0.09	13
3	Rect.	5 fish	0.98	0.565	0.355	0.09	2
3	Rect.	50 fish	0.90	0.565	0.355	0.09	2

* = 7.5 kg elvers + air injection
ref 1 = Burrows and Chenoweth (1955)
ref 2 = Burley and Klapsis (1985)
ref 3 = this study
rect. = rectangular
const = constant

Burrows and Chenoweth (1955) considered that all three of the ponds they tested had serious hydraulic defects, but that the raceway upon adaptation may justify usage in terms of cost. The conclusion that can be drawn from this is that t_c/t_i values of 0.83 and 0.88 were not considered to represent a well-mixed situation, whereas a value of 1.11 was considered to indicate that improvement was required. Burley and Klapsis (1985) indicated no preferential range of t_c/t_i values but pointed out that some recirculation with the total elimination of dead areas occurred in tanks with a t_c/t_i ratio of greater than

1. Both studies implied that an increase in t_c/t_i was advantageous. For general comparison and qualification of mixing, a minimum t_c/t_i value of approximately 1, which indicates tanks with no dead areas, can be considered adequate. It is likely that an upper limit for mixing exists, as determined by the stress imposed on the fish in a highly turbulent environment.

The improvement in mixing with decreased depth at a constant flow rate, shown in this study, confirmed similar conclusions drawn by Burley and Klapsis (1985). They however achieved an increase in mixing with increased stocking density of eels (Anguilla anguilla). This was not found in this study, in which juvenile turbot were grown. There were two possible explanations for this apparent disagreement. The greatest stocking density, of 50 fish $tank^{-1}$, used in this study was equivalent to 20.36 kg m^{-3}, which was less than the lowest density, of 22.1 kg m^{-3}, used in the eel study. So turbot stocking densities may not have been adequate to produce changes in mixing patterns. Secondly, eels are a more active fish than turbot, which tend to lie motionless on the tank floor. Casual observations indicated that after an initial period of movement, at the time of first appearance of the tracer into the tank, the turbot quickly settled down and moved little throughout the experiment. Other casual observations also confirmed that eels without overhead cover, particularly in high stocking densities, are almost

constantly moving and therefore stirring up the tank. It should be noted that at high stocking densities the volume of water will be appreciably less than at low densities, due to water displacement by the bodies of the animals.

Several conclusions can be drawn from this study. Though the results refer only to the particular conditions detailed in this study, certain principles may be more generally applicable. Decreases in residence time resulted in significant improvements in mixing. The reduction of depth in the tanks was a means of reducing residence time with no increase in flow rate. This would be particularly useful in situations where the quantity of water available was limited. Should flow rate be reduced to allow for reductions in water depth, no improvements in mixing will result unless residence time is also decreased. Increased flow rate into a tank, with no alteration of depth would be expected to increase mixing. There are limitations to the extent that alterations of depth, residence time and flow rate will have on mixing. A reduction in depth below a certain value will not improve mixing further and similarly an increase in flow rate above a value will produce negligible mixing benefits. These depth and flow rate values will vary with the design and management of each system.

Following chapters will examine the importance of mixing in the determination of water quality and various biological parameters.

5.7 SUMMARY OF CONCLUSIONS

Depth - constant flow rate:

1. Mixing improved with decreased depth of water in the treatment tanks within the range 9 - 18 cm.
2. A depth of between 2.89 cm and approximately 9 cm was required to minimise or eradicate dead volume in the treatment tanks at 2 l min^{-1} flow rate.

Depth - constant residence time:

3. Mixing was not influenced by depth changes, within the range 6 - 18 cm, with constant residence time and variable flow rates.
4. Residence time probably influenced mixing more than flow rate within the depth range 9 - 18 cm.

Flow rate:

5. Within the range 0 - 13 l min^{-1}, increased flow rate or decreased residence time, at a constant depth, improved mixing.
6. In a 9 cm deep tank, increased flow rates above 2.64 l min^{-1} produced only small improvements in mixing.

Stocking density:

7. Within the range 0 - 50 fish $tank^{-1}$ (equivalent to a mean stocking density of 0 - 1840 g m^{-2}) stocking density did not significantly influence mixing in treatment tanks with a depth of 9 cm and flow rate of 2 l min^{-1}.

Optimum:

8. A water depth of between 2.9 - 9 cm and a flow rate of 2.6 l min^{-1} at the deepest depth of this range produced the optimal hydrodynamics in the treatment tanks tested.

CHAPTER 6

HYDRODYNAMIC ASPECTS PART 2

FLOW VISUALIZATION

6.1 INTRODUCTION

Section 5.1 indicated that there are three main methods of studying the hydrodynamics of aquaculture tanks: residence time distribution, described in Chapter 5; flow velocity determinations, reported in Chapter 7; and flow visualization, described in this chapter. A study of hydrodynamics has, in this study, been used to provide data quantifying the water mixing within tanks controlled by various management regimes. The influence of the hydrodynamics on water quality and biological parameters was then investigated. Both residence time studies and flow rate determinations produce quantifiable data for comparisons with water quality and biological parameters. Flow visualization studies are more useful for qualifying flow patterns rather than quantifying the extent of mixing or the velocity of flows. For this reason visualization studies have a limited application in a study of this type, but they do have several advantages.

Flow distributions within a tank, no matter how simple its design, are often complex. Methods of quantification of the flows and mixing, invariably simplify the situation. Levenspiel (1966) stated that the

only way to predict the exact behaviour of a chemical reactor is to follow all molecules as they pass through the vessel, which is impractical. A method, such as the study of residence time distributions, yields only adequate rather than total information for the design of nonideal reactors, eg. aquaculture tanks. Flow visualization however, can clearly indicate the actual movement of water within a tank. All three methods of examining the hydrodynamics of a tank are effectively simplifications, but each method simplifies the situation in a different respect. A combination of these techniques should then reveal aspects of tank mixing which may not be determined by just one method. For this reason a flow visualization study was conducted, but not in great detail, because of the general nature of the results gained.

Flow visualization, as a method for determining the distribution of flows in vessels has been used for many years. The procedure involves the addition of a traceable material into a vessel, or an area of a vessel, so that the movement of the material may be observed. The tracer may not necessarily be: in the same phase as the fluid, such as the use of suspended plastic particles (Vogel and Feder, 1966) or electronically produced hydrogen bubbles (Vogel and LaBarbera, 1978); visible to the naked eye such as radioactive isotopes, eg. P^{32} (Bergman, 1958); used primarily to indicate the movement of the fluid, but may instead be employed mainly to indicate the movement of the

tracer itself, such as the use of gibsonite particles to simulate waste food deposition (Zielinski et al. 1976; 1978).

The majority of tracers are however clearly visible, coloured liquids used to indicate the movement of the carrying fluid. Section 5.4.1 indicates the tracers which have been previously used for residence time studies. Tracers used specifically for flow visualization included the dyes: rhodamine B which produced a bright gentian colour (Vogel and LaBarbera, 1978; Rosenthal et al., 1982); gentian violet (Burrows and Chenoweth, 1955); fluorescein sodium (uranine) which appeared orange, or green at low concentrations (Haskell et al., 1960; Vogel and LaBarbera, 1978); potassium permanganate crystals (Chamberlain, 1976). Crisp and Southward (1956) described the use of carmine, graphite, clay, starch and milk for flow visualization. The use of cows milk against a black background, was considered preferable to artificial emulsions. A problem associated with using milk in aquaculture situations is that it forms a film on tank and pipe surfaces, to the detriment of tank hygiene. In low concentrations it does however have the advantage of being nontoxic. Goldish et al. (1965) introduced a tracer into a vessel which turned into a dark blue colour when exposed to a burst of bright light. The colour could be detected visually or by colo rimetry.

Water quality parameters, such as DO, total ammonia

and nitrite concentrations, pH and biological oxygen demand, have been used to determine flow within tanks (Rosenthal et al., 1982; Burley and Klapsis, 1985; Rosenthal et al., 1985).

Denbigh et al. (1962) and Danckwerts and Wilson (1963) described the use of a time reaction for the study of flow distributions. Two solutions, continuously mixed prior to induction into the vessel, changed colour a definite period after mixing. The latter authors suggested that this method, which sets up a fairly stationary pattern of colour change, is preferable to the momentary impression given by dye injection. The reaction described, between persulphate and iodide ions yielding iodine, is not however suited to use in systems containing live animals because of the toxicity of the reagents.

Other studies including MacDonald and Piret (1951), Burrows and Chenoweth (1970) have used flow visualization techniques.

The tracer for flow visualization studies is normally introduced into the vessel in a short burst of varying duration, primarily dependant on the size of the vessel, flow rate and the tracer minimum detection concentration. In flow visualization studies it is the boundary, between the fluid containing the tracer and the fluid with which no tracer has mixed, which is of primary importance. It is this aspect of the study which distinguishes the use of

tracers for flow visualization and that for residence time determinations. The latter method indicates the rate at which a tracer exits the vessel, so the investigation proceeds for a period after the tracer has become fully mixed. Flow visualization studies investigate movement of the tracer into new areas of the vessel and so end when total mixing has been achieved. Alternatively a constant flow of tracer can be introduced into a vessel to produce a streamline effect (Vogel and LaBarbera, 1978). This method requires that the tracer concentration in the vessel other than the streamlining region does not become so great that the streamline cannot be distinguished, for reasons described above.

Tracer concentration or position may be determined by: eye, still or video photography, when using coloured dyes or suspended particles; spectrophotometrically; or by chemical analysis or physical measurement in the case of water quality parameters. The latter two methods require the sampling of quantities of fluid at specific times and specific locations within the vessel and may alter the system being sampled.

Information on flow velocities at points within the tanks and limited information regarding water quality gradients in the tanks, were available from other investigations (Chapters 7, 8 and 9 respectively). A general indication of flow patterns determined by visualization studies was therefore required.

6.2 OBJECTIVES

1. To illustrate flow distribution patterns within the treatment tanks.
2. To compare these patterns with results of other methods of determining flow distributions and mixing within the treatment tanks, so that a greater understanding of the hydrodynamics and their influence on water quality and biological parameters (discussed in Chapters 8 - 11) is achieved.
3. Confirmation or rejection of flow patterns indicated by other methods of analysis.

6.3 MATERIALS AND METHODS

For reasons indicated in section 5.4.1 the blue food colourant dye (COB) described in that section was used to visualize flows in the treatment tanks. The study was carried out in conjunction with the residence time study. A description of the equipment and method used is given in sections 5.4.2. and 5.4.4 respectively. After injection, the dye was observed and a note was taken of its distribution at intervals after injection. Dye location was recorded by hand drawings at the time of the observations. This was considered sufficiently accurate for the purposes of this study. For reasons described above, the visualization of flows under all treatment conditions was not carried out because only a general indication of flow pattern in the tanks was required.

6.4 RESULTS

Figure 6.1 indicates representative dye distributions in the treatment tanks. Figure 6.1a illustrates the flows in a shallow tank, filled to a depth of 6 cm, with a flow rate of 1.14 l min^{-1} and ideal mean residence time of 10.84 min. Between 0 - 2 min the dye passed into the tank, via the inlet spray pipe, fairly evenly across the full width of the tank and at all depths. The dye front progressed along the length of the tank, retaining an even front boundary approximating plug flow. Behind the front, the dye was evenly mixed. At 2 min, water containing no dye appeared at the inlet spray pipe. Side eddies moving upstream along the tank sides caused this area of low dye concentration water to be carried into the centre of the tank. Further influent water mixed evenly with the existing tank water until at 4 min an homogeneous mix was achieved.

Figure 6.1b indicates the flows in a tank filled to a depth of 9 cm, with a flow rate of 1.0 l min^{-1} and ideal mean residence time of 18.54 min. At 0.5 min a distorted plug flow was apparent. Between 1 - 2 min the flow along the side walls was greater than that in the central region where back mixing mainly along the bottom, produced a well mixed region behind the advancing front. Between 2 - 3 min the flows along the side walls met at the effluent end of the tank, resulting in a fully mixed fluid.

Figure 6.1: Flow visualization study - dye distribution in the treatment tanks

(time in min)

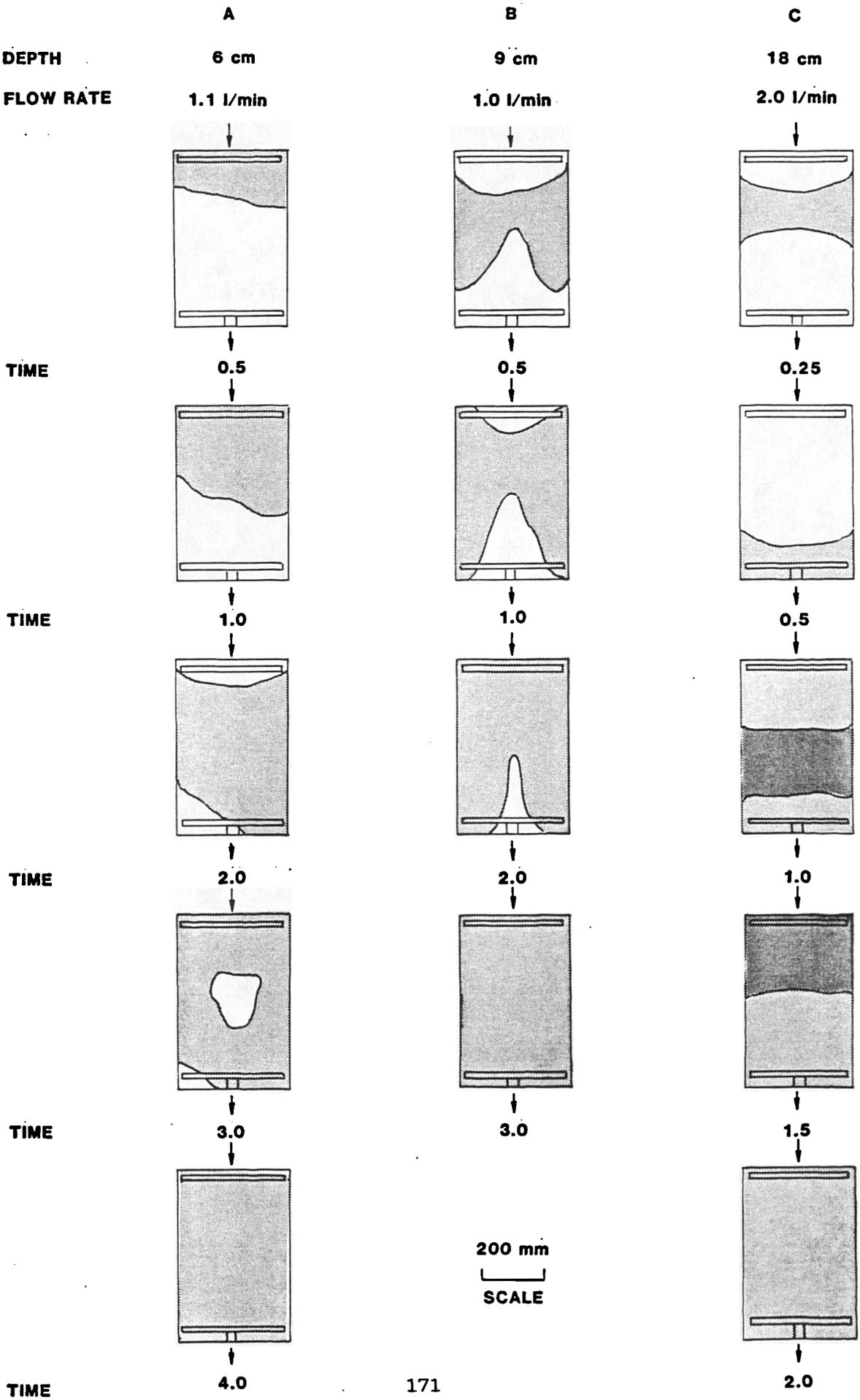


Figure 6.1c demonstrates the flows in a tank filled to the maximum depth tested of 18 cm, with a flow rate of 2.0 l min^{-1} and an ideal mean residence time also of 18.54 min. For the first 0.5 min the dye moved as a plug along the length of the tank, mainly at the upper depths. Elongation of the plug occurred as dye at the tank floor travelled more slowly than dye in the upper layers. Between 0.5 - 1.5 min the dye plug reflected off the effluent end of the tank and passed back up the tank mixing with new influent water as it did so. Mixing of plug water with influent water produced an homogeneous solution within the tank by 2 min after the initial injection.

6.5 DISCUSSION

Although the three examples described were not all of the same flow rate or residence time, thus limiting more quantitative comparisons, the effect of depth on the flows in the tanks was apparent. In the shallowest tank the initial plug flow nature of the dye distribution soon extended along its full length as the dye front passed through the tank. Dye also remained behind to mix with incoming water containing no dye. The probable cause of this situation was due to two phenomenon, called the surface tension and boundary layer drag effects, which have been described by Burrows and Chenoweth (1955) and Carstens (1968). Surface tension, at the free surface of a fluid, slows down the velocity of the fluid. The effect

decreases with increasing distance from the surface. Boundary layer drag occurs because the velocity of the fluid at a wall must be zero, so producing a velocity gradient increasing away from the boundary (Carstens, 1968). This is despite the smooth walls and floors recommended by Larmoyeux et al. (1973) and the orientation of boundaries parallel to the streamflow, recommended by Zielinski et al. (1974). Larmoyeux et al. (1973) also found frictional torque in circular ponds due to the boundaries, though Burley and Klapsis (1985) studying round cornered 1 m^2 tanks discovered a relatively small energy loss due to wall surface friction drag, probably because their depths and flow rates were greater than those in this study. Burrows and Chenoweth (1970) stated that as the depth of water in a tank increases, the water velocity along the bottom decreases and the average velocity occurs at 0.4 times the depth, 2.4 cm in the 6 cm deep tank described. An ideal plug flow situation is not possible in practice. Molecular and turbulent diffusion (Levenspiel, 1966) would be expected to distort the dye plug even if frictional boundary forces were negligible.

At the maximum depth tested (18 cm) a more plug flow situation than that which occurred in the 6 cm deep tank, was evident. This may have been due partly to the increased flow rate, but such an increase was more likely to have caused the reduction in time for the plug to pass along the tank, rather than maintaining the coherency of the plug.

The 9 cm depth situation can be regarded as intermediate between the two examples above, if allowance is made for the low flow rate. A plug flow was approached shortly after introduction of the dye into the tank, but frictional drag on the tank floor, exacerbated by the lack of momentum energy of the fluid due to the low flow rate, held back and distorted the dye front.

The area available to cause surface tension drag was the same in all tanks, so the predominant influence on the flow distributions appears to have been boundary layer drag. In shallower tanks the surface area to volume ratio was greater than in deeper tanks with a larger volume. The flow visualization results of this study indicate that at shallower depths, boundary layer drag becomes more important than in deeper tanks, particularly at the low flow rates employed in these experiments. This caused flow separation and eddies to occur, which increased the mixing of new influent water. In deeper tanks a more plug flow situation was approached because a greater volume of water entering the tank did not approach the boundaries and so was able to maintain its momentum towards the outlet end of the tank. At the effluent end of the tank however, the method of water extraction was inadequate to completely remove the plug of water which passed down the tank. A considerable degree of reflection off this end wall then caused the plug to become mixed into the fluid in the remainder of the tank.

Such flow patterns have implications for turbot lying on the bottom of the tank. In shallow tanks which are well mixed, a more constant water quality would be expected, with fewer periods of, or locations containing, either very good or very poor water quality. The distribution of fish in the tanks should be more uniform and the variation of biological parameters, such as growth rate, may consequently be smaller. The increased flow separation at the tank floor, and greater flow speed, as described by Burrows and Chenoweth (1970), may help to clean the tank floor and resuspend pellets. Alternatively more energy may be expended by the fish to maintain their position against the mixing eddies.

In deeper tanks the passage of a plug of water in both directions along the tank suggests that flows in different directions occur at different levels in the tank. This leads to the possibility of the sort of dead areas described by many authors including Burrows and Chenoweth (1970) and Rosenthal et al. (1982). Provided that the fish remain on the tank floor, which is usual with turbot, a central dead area may not be a problem, however if the region extends to the tank floor, poor quality water may come into contact with the fish. This, combined with the lower flow velocities at the tank floor, predicted by Burrows and Chenoweth (1970), in the deeper tanks, may result in poorer water quality conditions for the fish on the bottom of such tanks.

It should be noted that the flow visualization studies were conducted with no fish present. It is expected that with fish present the flow distributions may have been different, the extent of the difference dependent on stocking density. However with inactive fish lying on the bottom, ie. turbot, this variable may not be as important as in the situation with pelagic fish described by Rosenthal et al. (1985).

6.6 CONCLUSIONS

1. Mixing appeared greater in shallower tanks.
2. Boundary frictional drag was an important factor determining flow in shallow tanks.
3. The possibility of dead areas in deeper tanks resulting from flows in opposite directions at different depths was likely.

CHAPTER 7

HYDRODYNAMIC ASPECTS PART 3

FLOW VELOCITY AND FISH BEHAVIOUR

7.1 INTRODUCTION

Chapter 5 described the quantification of mixing in the treatment tanks and results in Chapter 6 were used to illustrate the flow dynamics in the same tanks. Both these methods were suited to the determination of the hydrodynamics of tanks of different working depths. Another variable associated with water depth changes in a tank is flow rate. During growth experiments, described in Chapters 8 and 9, flow rate was adjusted to maintain the same residence time in half the total number of treatment tanks. A consequence of the maintenance of a constant residence time at different depths of water was that flow rate was not controlled. In both growth experiments replicate treatments were conducted, with either flow rate or residence time the same at different water depths, so that differences in biological or water quality parameters between treatments could be attributed to either changes in depth, flow rate or residence time. Other Chapters describe the effect of flow rate on fluid mixing (Chapter 5), growth and fitness of turbot (Chapters 8 - 10) and on water quality (Chapters 8 - 10). Experiments described in this chapter have been conducted to investigate any direct effects of flow rate, on the behaviour of the turbot and to establish which, if any,

hydrodynamic consequences of flow rate into the tanks, such as local flow velocities and flow directions within the tanks, influence the turbot. The data obtained were also compared with the results of flow visualization studies (Chapter 6) and residence time mixing studies (Chapter 5), to improve the understanding of fluid movement within the study tanks.

Compared with flow visualization studies, few investigations have been conducted in which flow velocities have been quantified to indicate the hydrodynamics in aquaculture tanks. Burrows and Chenoweth (1955 and 1970) detailed flow velocities in circular and rectangular tanks respectively, to indicate regions of poor mixing. The methods of measurement were not specified, but it is thought that a dye tracer was used. In a study of factors affecting flow patterns in Macrobrachium rosenbergii larval tanks Zielinski et al. (1976) used a miniature current meter to measure flow velocities. An interesting aspect of the study was that the deposition of "Gibsonite particles" was compared with the flow velocities, so that regions of food settlement could be estimated. Vogel and LaBarbera (1978) reviewed various methods for the determination of flow velocities. These included timing the passage of a tracer such as Rhodamine B, Evans blue, milk, colloidal carbon, plastic particles, aluminium dust or hatched brine shrimp (Artemia salina) using long and multiple exposure photography. Other methods listed were the use of a pitot tube and

manometer, and heated thermistor probes which lose heat in relation to the local water speed, also described by LaBarbera and Vogel (1976). In a comprehensive study of hydrodynamic mixing within a 1 m^2 elver (Anguilla anguilla) tank, Burley and Klapsis (1985) constructed velocity maps from data obtained using the same stream-flow meter that was employed in the present study.

7.2 OBJECTIVES

1. To quantify the flow patterns within a treatment tank.
2. To study the effect on flow patterns, of altering flow rate into the tank.
3. To investigate the influence of flow velocity (speed and direction) on fish position, movement and orientation.

7.3 MATERIALS AND METHODS

7.3.1 Summary of method

The experiment was conducted in five stages:

1. A treatment tank was adjusted to hold water to a depth of 9 cm, at a range of six inflow rates between 0.5 - 13.0 l min^{-1}.
2. The velocity of slow flows, at positions in a defined grid of locations near the floor of the tank, was measured using a dye tracer.

3. The velocity of faster flows at the same defined grid of locations was measured using a flow meter.
4. Having established the flow patterns in the tank at different flow rates, a single turbot was placed into the tank. The fish was observed using a video camera and recorder, and its location, orientation and movement was noted each minute for 60 min. The experiment was repeated with four other single fish at each of the six flow rate treatments.
5. Inflow rate, local flow velocities and flow directions were compared with fish location, orientation and movement.

7.3.2 Experimental regime

A treatment tank described in section 4.1.2 was used throughout this flow velocity study, so that some comparison of results with other aspects of the tank culture of turbot, described in other chapters, could be made. A grid of 77, 5 cm squares was marked out on the floor of the tank. The column and row grid coordinates (A1 - G11) were written in the edge squares (Figures 7.1-7.6).

The minimum stable depth of water in the tank, which could be maintained at flow rates of 0.5, 1.0, 2.0, 4.0, 8.0 and 13.0 l min^{-1}, determined by trial and error, was 9 cm, due to limitations on the quantity of water which could be removed from the tank. A depth of water which

Figure 7.1: Flow velocity profile and distribution of settled fish - 0.5 l/min inflow

(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)

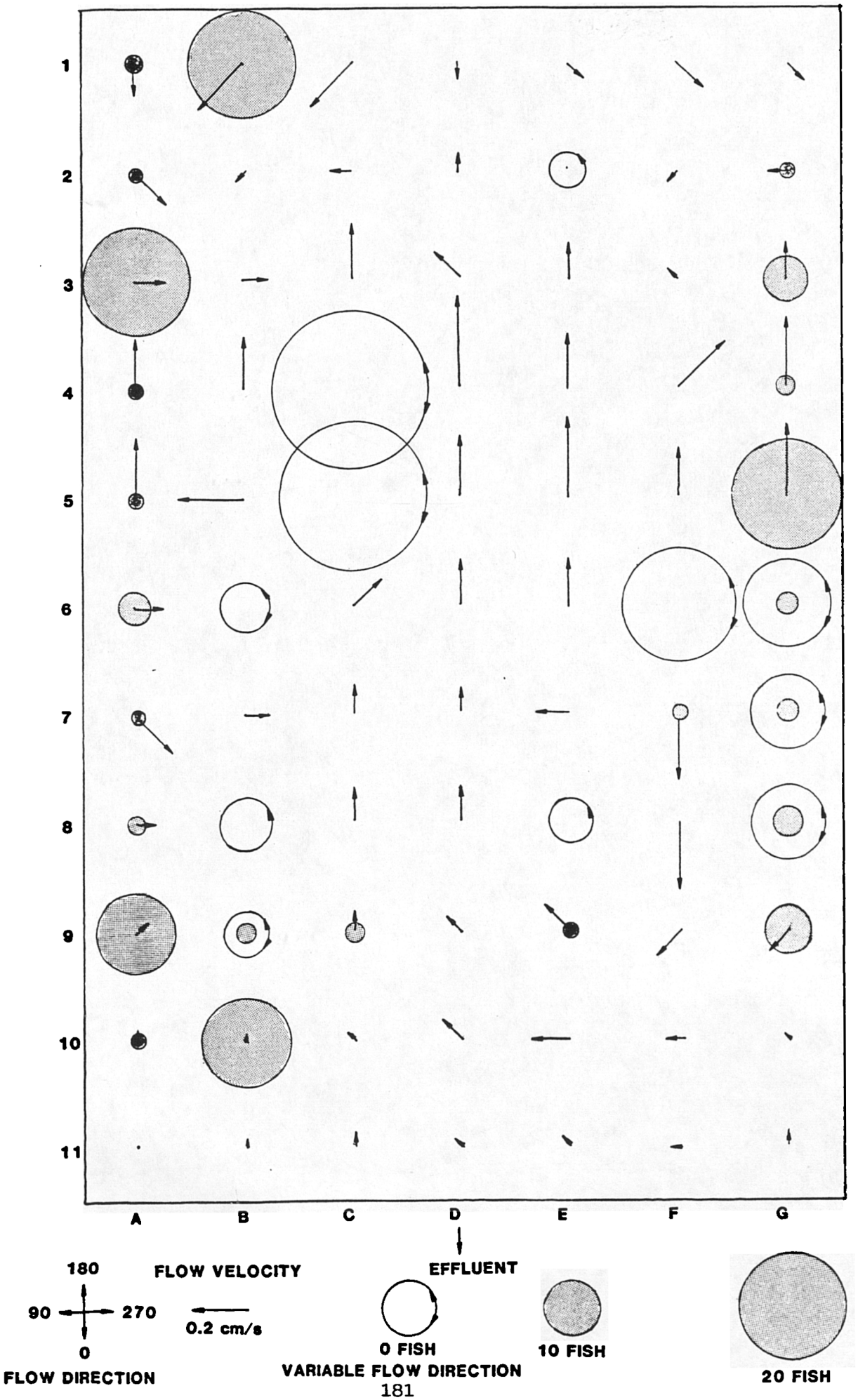


Figure 7.2: Flow velocity profile and distribution of settled fish - 1.0 l/min inflow
(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)

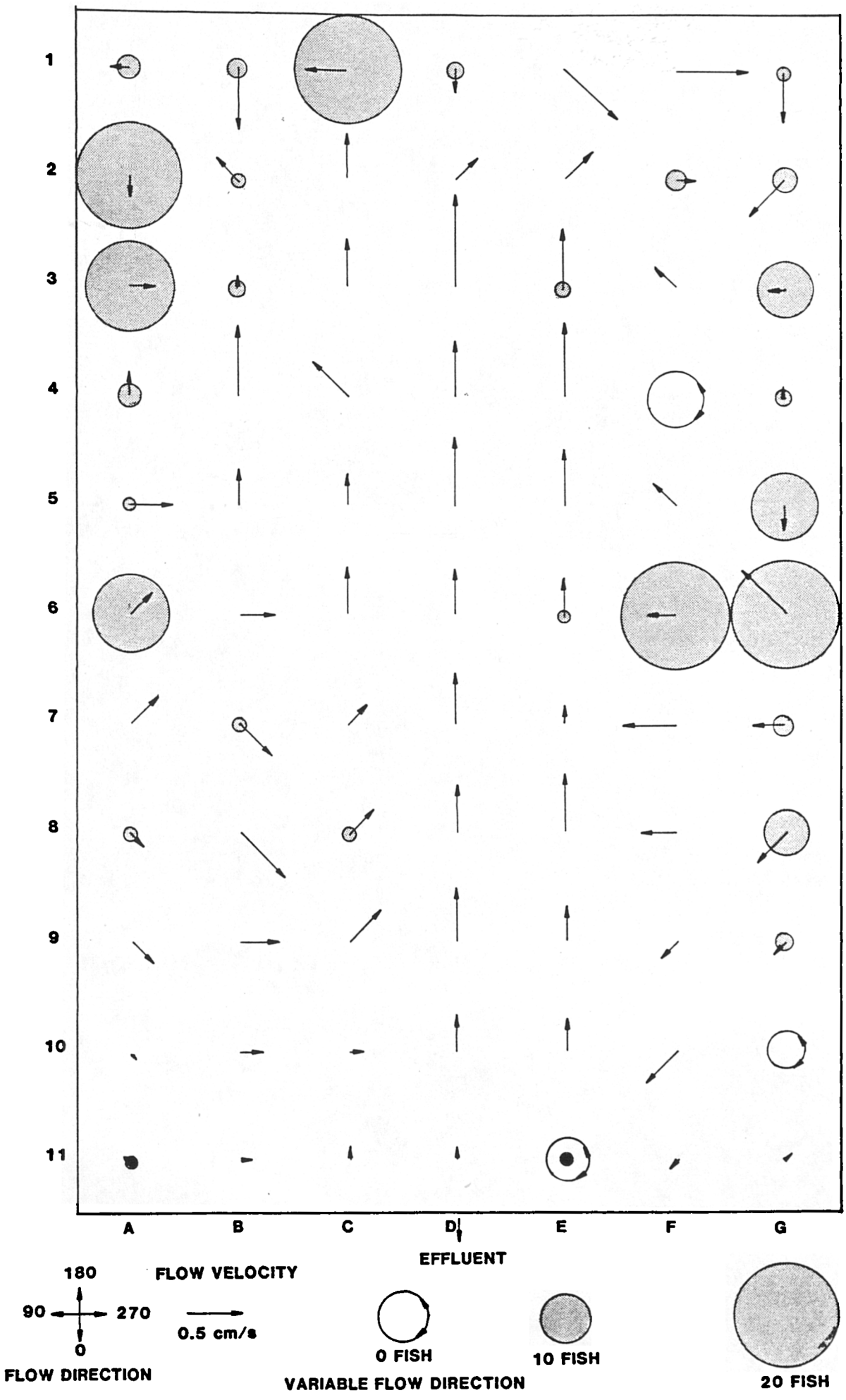


Figure 7.3: Flow velocity profile - 2 l/min inflow

(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)

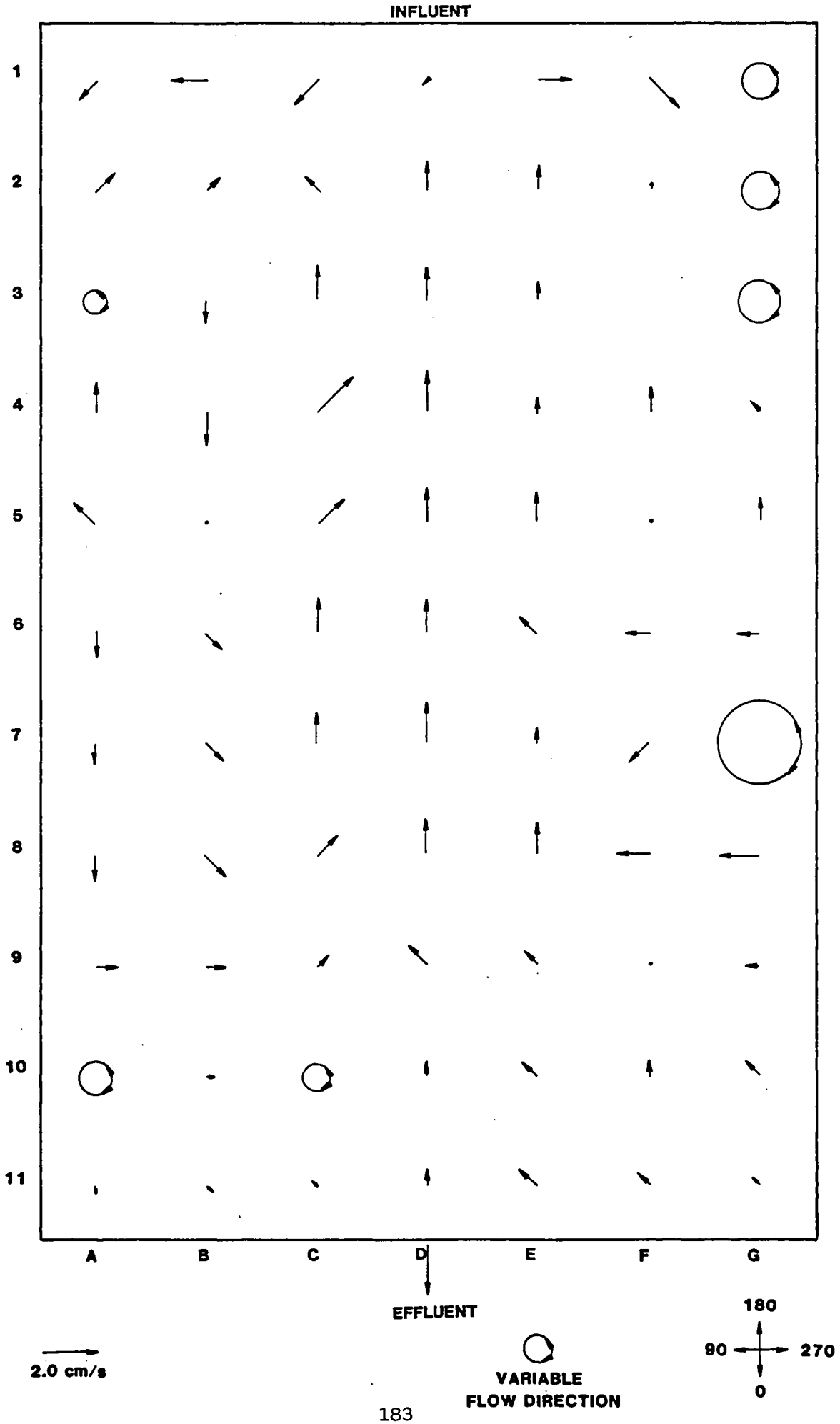


Figure 7.4: Flow velocity profile - 4 l/min inflow

(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)

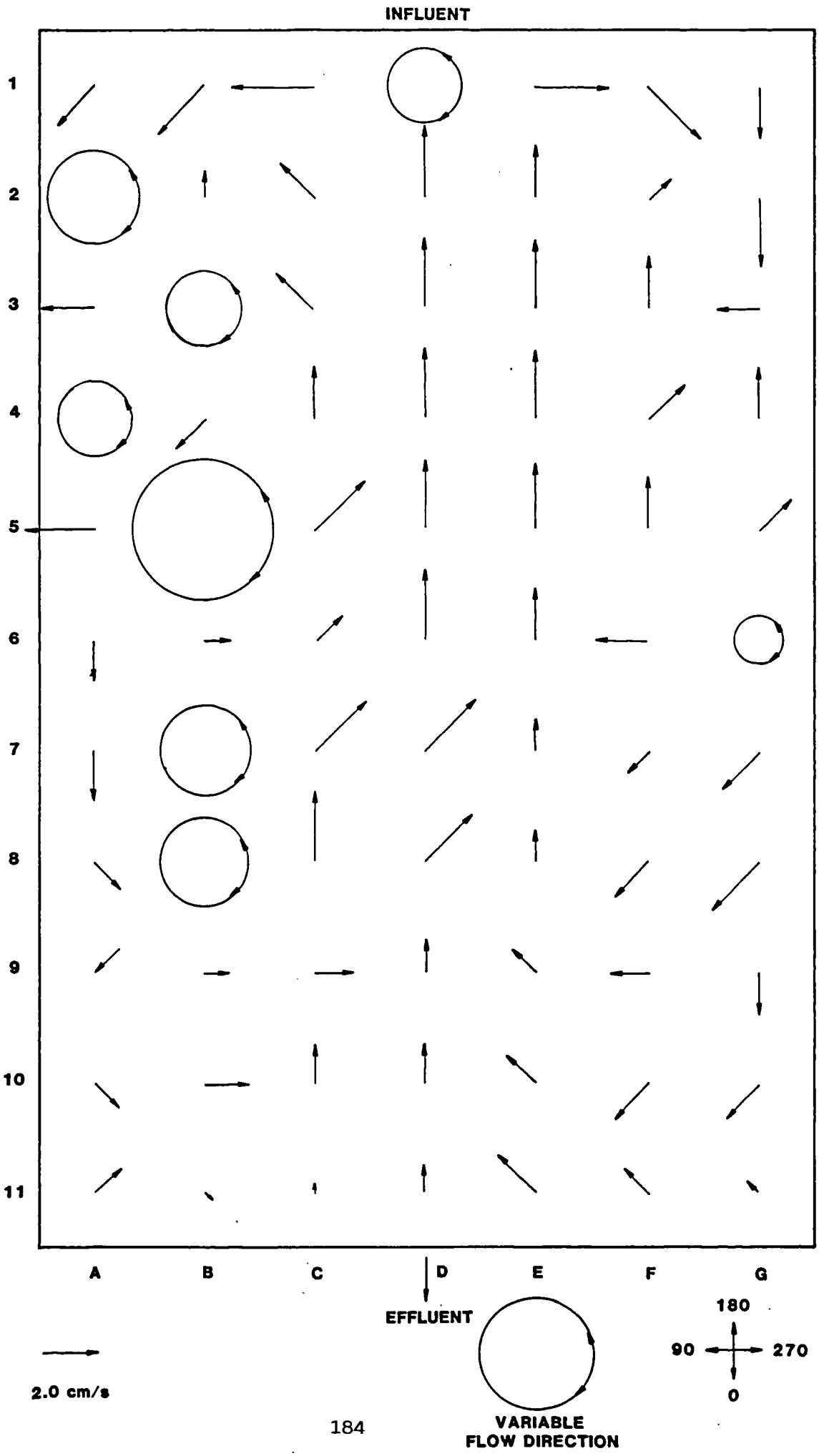


Figure 7.5: Flow velocity profile and distribution of settled fish – 8.0 l/min inflow

(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)

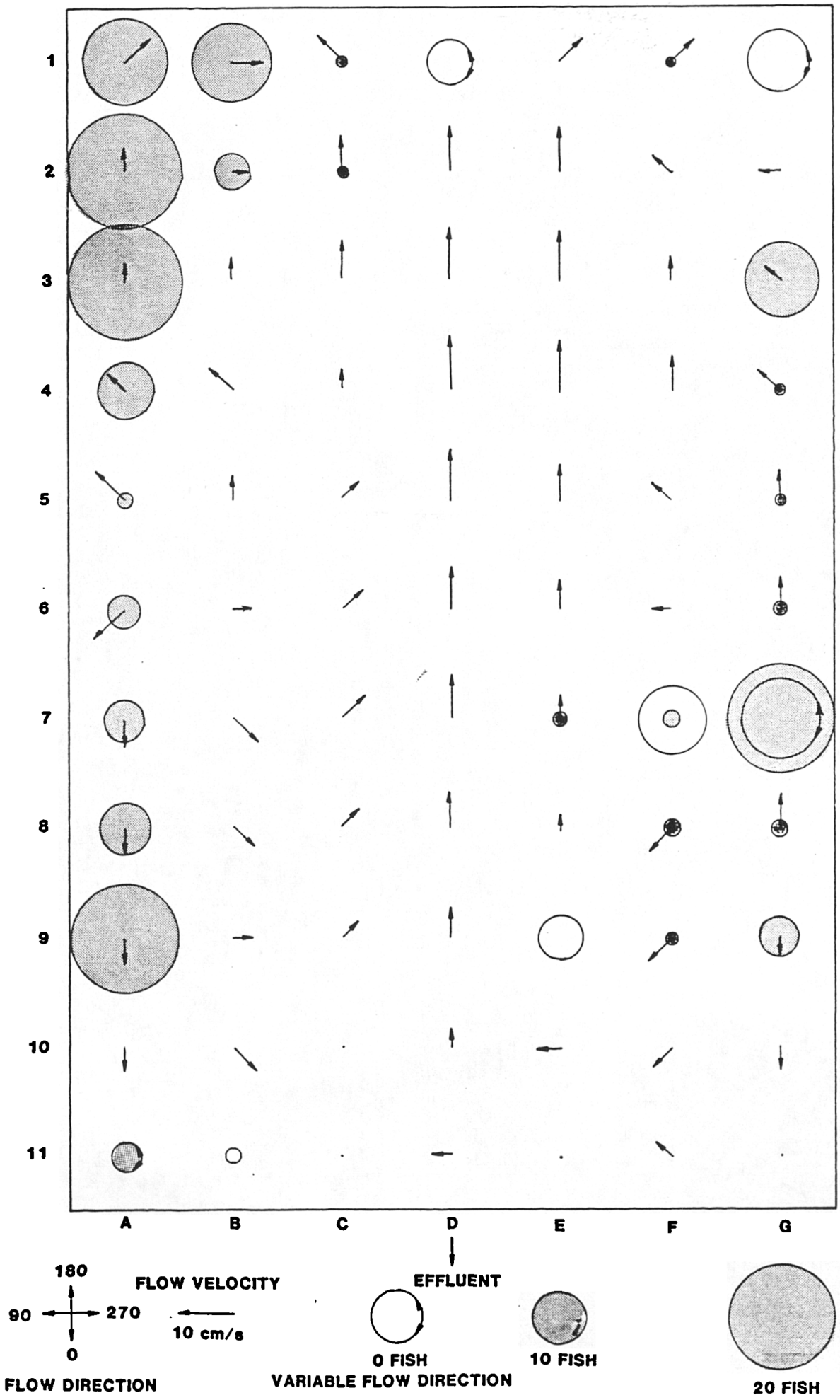
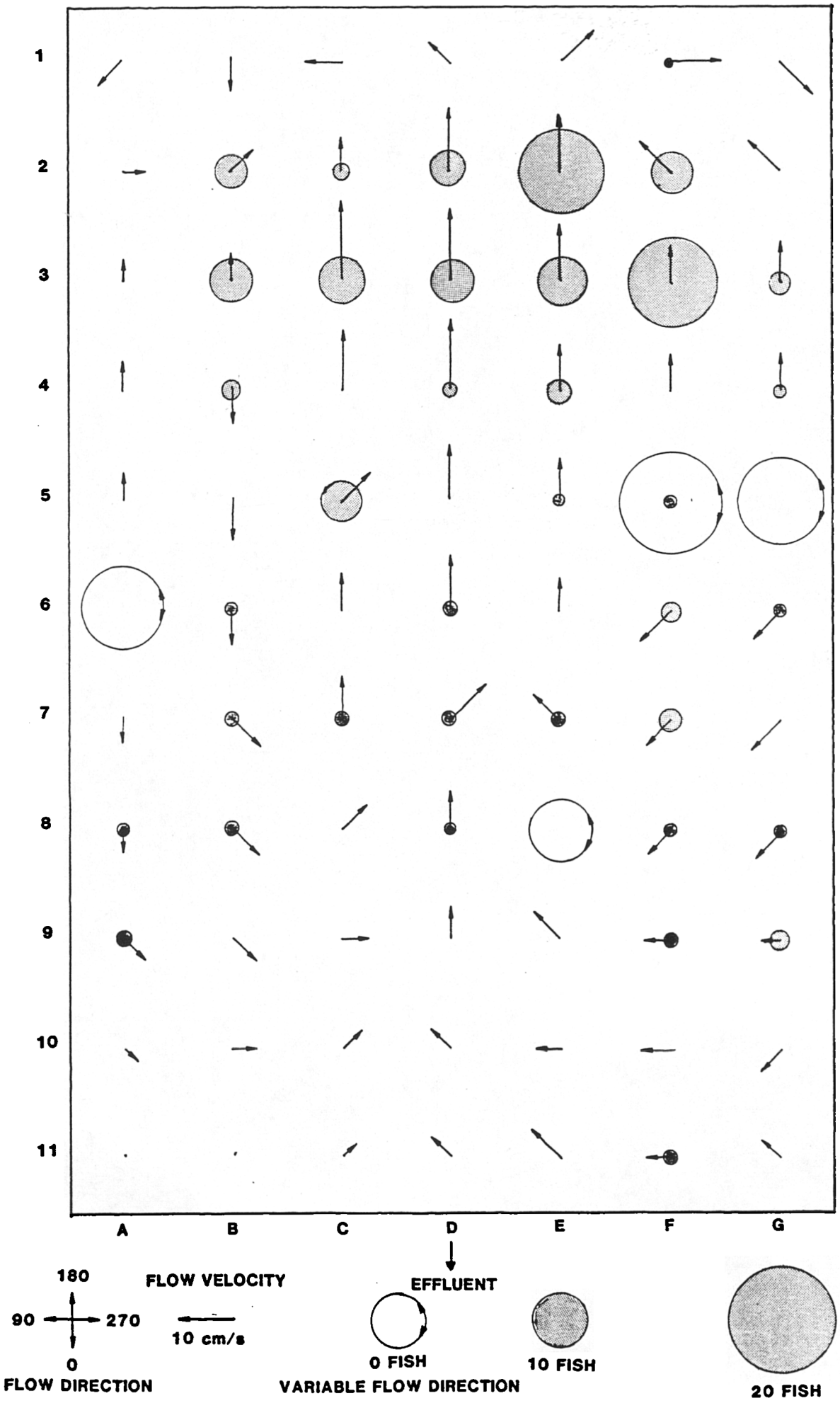


Figure 7.6: Flow velocity profile and distribution of settled fish - 13 l/min inflow
(tank: 565 x 365 mm, water depth 9 cm, velocity measurements 1 cm above tank floor)



was as shallow as possible was adopted because it was expected that more easily definable, laminar flows with less vertical movement of fluid, would occur in the shallower tanks. This depth of water, and therefore volume, was common to a depth adopted in other water depth experiments (Chapters 5, 6, 8 and 9). The recirculation system (Figure 4.1) described in section 4.1.1, filled with seawater, was used.

7.3.3 Determination of flow velocities using tracers

The correct flow rate into the tank was regulated by the inflow valve. The standpipe height was adjusted accordingly to obtain the correct water depth in the tank. A period of 1 h was allowed for the establishment of a steady state flow pattern undisturbed by changes in inflow rate and standpipe height adjustments.

The tip of the needle on a hypodermic syringe was positioned 10 mm above the middle of the tank floor grid square to be studied. The length of the needle was sufficient to avoid immersion of the hypodermic reservoir, so obstructing flow patterns as little as possible. The syringe was maintained perpendicular to the tank floor and care was taken to avoid unnecessary movements of the needle, which would also have influenced water flow. COB dye, described in section 5.4.1 was injected into the tank. The rate of dye injection was minimised to ensure that the resulting direction of flow of the dye was not

influenced by the force of ejection out of the syringe. The time taken for the dye to travel a horizontal distance of 2.5 cm, ie. from the injection needle to the perimeter of a 5 cm diameter circle, drawn within the 5 cm grid square on the tank floor, was noted. The direction of travel of the dye, determined by the point the dye crossed the grid circle, was measured in degrees to the nearest 45 ^{o}, relative to the long axis of the tank from the inlet to the outlet (Figure 7.7). Due to the variation in mixing within the tank, no greater accuracy of direction measurement was possible.

Due to variations in flows caused by flow separation within the tanks, five flow direction and velocity measurements were taken at each of the 77 (11 x 7) grid squares. The flow velocity at each grid square was calculated by the equation:

$$v\ (cm\ s^{-1}) = \frac{d\ (cm)}{((t_1+t_2+t_3+t_4+t_5)\ /5)\ (s)}$$

where: v = flow velocity (cm s^{-1})

d = distance dye travelled (2.5 cm)

t = time for dye to travel d cm

Direction of flow was calculated as the mean of the five replicates, to the nearest 45 ^{o}. Flow direction was noted as variable if the range of replicate flow directions was greater than or equal to 180 ^{o}.

7.3.4 Determination of flow velocities using a streamflow meter

Local flow velocities resulting from flow rates into the tank greater than 4 l min^{-1} were not determined using tracers because the time taken for the dye to travel 2.5 cm was too short to be measured accurately. An increase in the distance over which the dye travelled, so increasing the time measurement, would have resulted in the inclusion of flow velocities and directions relevant to other grid squares in the resultant velocity.

A Nixon Instrumentation Streamflo Probe, type 403 and indicator were used to determine local flow velocities. The 5 bladed rotor, held within a 15 mm diameter cage responded to velocities greater than 2.5 cm s^{-1}. Output from the indicator, in revolutions per second (Hz) of the probe rotor, was converted into linear velocity (cm s^{-1}) using a probe-specific calibration curve supplied with the probe.

Due to the complicated nature of the flows in the treatment tank and the fact that the probe measured flows along only one axis at a time, a method was developed which provided a quantitative estimate of flow velocities in the tank.

Using the same method described in the previous section, flow rate into the tank was regulated, depth was

fixed and a steady mixing state was achieved.

The probe was lowered into the tank water and the bottom of the rotor cage was allowed to rest on the tank floor in the middle of the grid square to be studied. Care was taken to hold the probe stem vertical and to reduce disruption of the flows by movement of the probe. The central axis of the probe rotor was orientated to point the probe along the longitudinal axis of the tank (0 ^{o} - 180 ^{o}, Figure 7.7). The number of revolutions per second of the rotor, integrated over a 10 s period, was noted. The probe was then rotated horizontally through 90 ^{o} to point along the transverse axis of the tank (90 ^{o} - 270 ^{o}, Figure 7.7) and the number of revolutions per second, integrated over a 10 s period, was again measured. This procedure was repeated a further four times at each grid square.

The mean velocity of the five replicates, along both axes, was then calculated. The probe did not however indicate which of the two possible directions along each axis the mean flow occurred. Dye tracer, in a method similar to that described in the previous section, was used to determine the direction of flow to the nearest 45 ^{o}. Knowing the approximate direction of flow, vector addition of velocities was calculated to indicate both the velocity of flow and direction, to the nearest 45 ^{o}. On those occasions where the dye tracer indicated that flow was along the longitudinal or transverse axes of the tank,

the velocity value along the same axis only was included in the calculation of mean flow velocity. The rejected mean velocity measurement along the axis perpendicular to the resulting flow was considered to be due to flow separation. This method was used to obtain flow velocity data comparable with behaviour results. Examples of this method of velocity calculation are described in Figure 7.8.

7.3.5 Behaviour experiment

A screen was built around the treatment tank to reduce the influence of external parameters on fish behaviour, such as sunlight direction. The same low light levels were maintained during all treatments. Movements and sources of vibration, with the exception of the recycle system pump, around the vicinity of the tank were reduced to a minimum. A video camera was attached to the roof screen above the centre of the tank. This was connected via a camera switching unit to a monitor and time lapse VHS video recorder located in a different room to that in which the treatment tank was located. The fish were therefore observed without disturbance. The output from the camera was passed through a timer unit which superimposed a digital clock onto the picture stored on video tape.

From a stock of 120 fish kept in the same type of tank with a water depth also of 9 cm and a stocking

Figure 7.7: Water flow and fish direction relative to the treatment tank

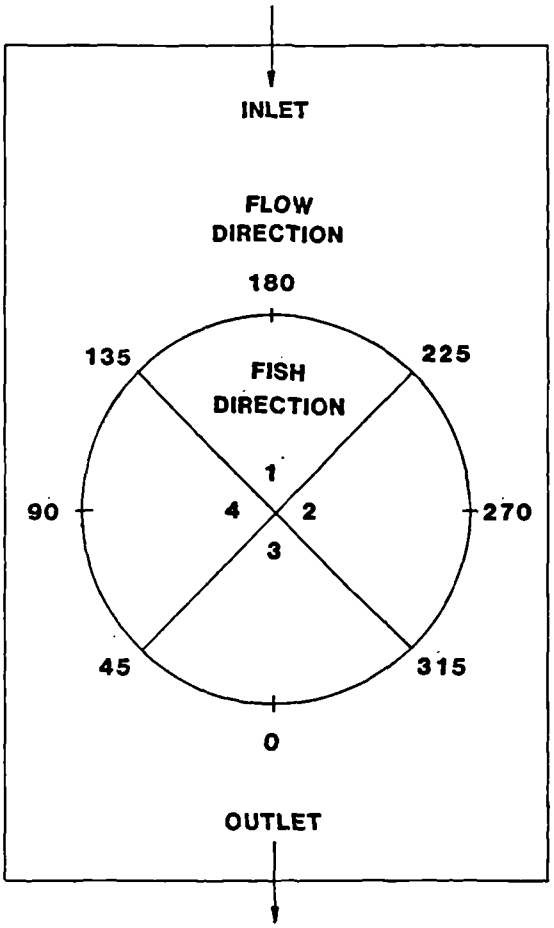
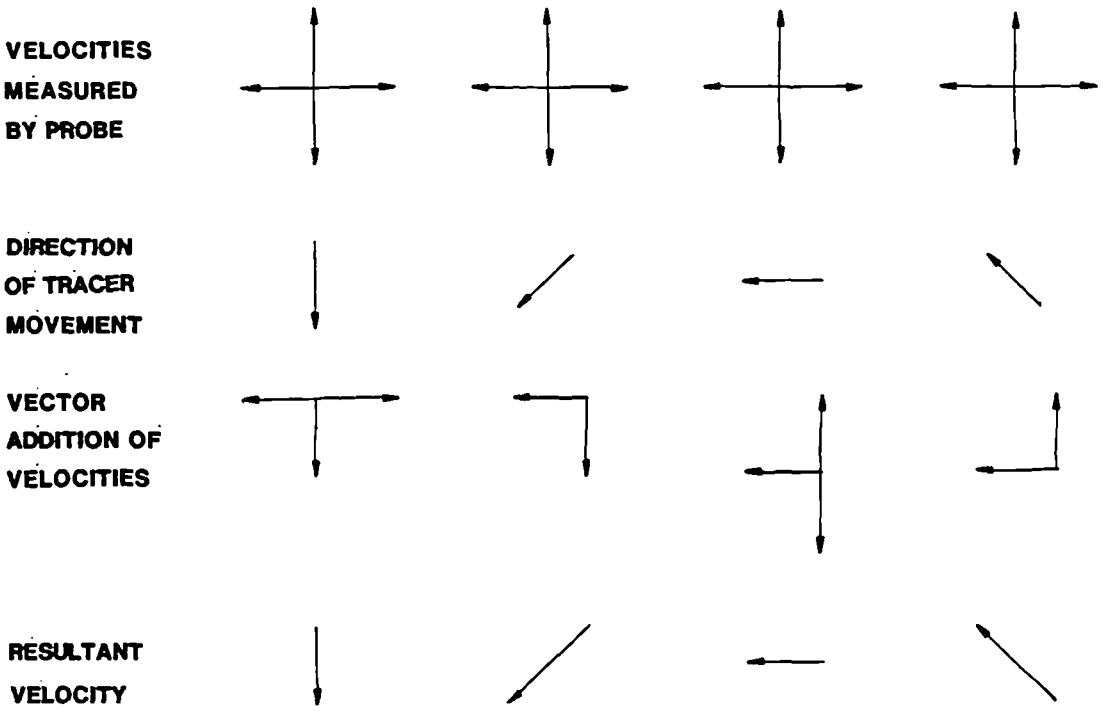


Figure 7.8: Flow velocity calculation examples (measured by flow meter)



density of 30 fish $tank^{-1}$, a random sample of five fish were chosen. These five fish were used in a random order, to reduce the influence of acclimation on behaviour, in each of the six flow rate treatments. This was considered preferable to using different fish in each experiment, from the full 120 stock fish. By using the same five fish, the influence of size on fish behaviour in different flows was reduced. The weight, length, pigmentation type and distinguishing features of each fish was noted at the beginning of the experiment, so that individual fish could be identified. Reasons for the use of solitary fish will be discussed in section 7.5.

The video recording was started and a turbot, sampled at random from the stock of five fish, was placed in the treatment tank in which flows had stabilised for 1 h after flow rate and depth adjustments. For a period of 60 min from the addition of the turbot into the tank, the location, orientation and movement of the fish was noted at 1 min intervals. The location of the fish was determined relative to the grid of 5 cm squares drawn on to the tank floor for the quantification of flow patterns (see sections 7.3.3 and 7.3.4). Orientation of the fish was determined as the direction in which the fish was pointing at the time of viewing. Low light levels and the method of time lapse recording used, adversely affected the definition of the video picture recorded, so it was considered that fish direction could only be determined reliably to a minimum of the nearest 90 ^{o} (Figure 7.7).

By comparison of the position of the fish immediately before and after the measurement time, the movement of the fish was determined. Fish were noted as either swimming or settled.

After 1 h the fish was returned to a holding tank on the same recycle system and another fish of the 5 stock fish was sampled and identified for behavioural analysis. After all five fish were separately placed in the treatment tank, the flow rate of the tank was altered and the experiment was repeated.

7.3.6 Analysis of data

Using the methods described in sections 7.3.3 - 7.3.5 the following comparable data was available describing each of the 77 grid squares at each of the six flow rate treatments: flow velocity, flow direction, number of fish present, number of fish settled and orientation of fish. For results from each of the six flow rates tested, and a combination of the data from all the flow rates, the following analyses were conducted.

The influence of flow velocity on flow direction at each grid square was investigated by plots, correlation and regression analyses, to establish if flows became more linear with increased flow velocity.

The distribution of fish within the tank with respect

to flow velocity and flow direction was investigated by plotting the preference of individual fish for each grid square which was indicated by the number of times fish were present at each grid square, against the flow velocity and flow direction at the same squares. Moving fish may have merely been passing through a square at the time of measurement and may not have selected that area to settle in, so the number of times fish settled in each square was plotted against the flow velocity and direction at the same squares. As a further measurement of fish location preference, the settled proportion of the total number of fish occurrences in each square, was plotted against flow velocity and flow direction for each square. All plots were quantified by correlation and regression analyses. It was possible that both flow velocity and flow direction together may have influenced fish location preference, so a multiple regression analysis, using the Minitab statistics package (Ryan et al., 1985), was calculated to examine the influence of these parameters on the total number, settled number and settled proportion of fish present at each square.

The mode direction the fish was facing was calculated, to estimate the orientation of fish to the flow direction at each square in which they were present. The angular difference between the mode direction the fish were facing and the flow direction, was calculated. This direction difference was plotted against flow rate and quantified by correlation and regression analyses.

To further study the influence of flow direction on fish location preference, the mean number of fish which settled on squares in which one of the nine possible flow directions, including "variable direction", was calculated, for each flow rate and plotted as circular bar charts.

The total number of times fish settled, total number of times fish were swimming and the mean flow velocity during each flow rate treatment was calculated. For each flow rate the total number of fish behaviour observations was 300 (60 x 1 min observations x 5 fish). The proportions of settled and swimming fish at each flow rate and at each mean flow velocity were compared using correlation, regression and chi-square tests.

7.4 RESULTS

Table 7.1 indicates the dimensions of the turbot used in this study.

Table 7.1: Turbot dimensions

Fish no.	weight (g)	length (mm)	pigm p/m
1	11.7	86	m
2	10.7	86	m
3	9.3	83	m
4	22.3	108	m
5	7.7	78	p

Flow velocity and fish behaviour results are shown in

Tables 7.2 - 7.6. Flow velocity profiles in relation to the number of settled fish in each grid square are indicated in Figures 7.1, 7.2, 7.5 and 7.6. Due to the use of inferior quality video cassettes, the definition of recordings of fish behaviour in the 2 and 4 l min^{-1} treatments deteriorated after playing in the time lapse recorder, which disrupted the oxide coating on the video tape. Behavioural recording for these two treatments were rendered unusable, though flow velocity and direction data were available.

A similar pattern of flow velocities is shown at all flow rates. The majority of flows were in the opposite direction to the overall flow from influent to effluent. Along the side walls of the tank, flows were less uniform and linear. In approximately square rows 6 - 11, flow direction was generally away from the side walls towards the effluent end of the tank. In squares 1 - 5, flow direction was also generally towards the centre of the tank, but towards the influent end. Flows near to both end walls were more variable in velocity and direction than other regions of the tank. Flow was generally directed away from the tank longitudinal centre line at the influent end and towards the centre line at the effluent end. All four corners of the tank were regions of the most variable flow direction, though at positions close to A6 and G6, mid-way along the side walls, flow direction was highly variable. A pronounced linear flow was evident along the centre of the tank, towards the

Table 7.2: Flow velocity and fish behaviour results - 0.5 l min^{-1}

grid position	flow velocity ($cm\ s^{-1}$)	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
A1	0.12	0	3	-	2	0.67
A2	0.17	315	7	45	1	0.14
A3	0.11	270	57	0	49	0.86
A4	0.19	180	5	90	2	0.40
A5	0.24	180	5	90	2	0.40
A6	0.10	270	5	90	5	1.00
A7	0.18	315	2	45	1	0.50
A8	0.07	270	6	-	3	0.50
A9	0.05	225	19	45	15	0.79
A10	0.04	180	1	90	1	1.00
A11	0.02	180	1	90	0	0.00
B1	0.24	45	60	135	60	1.00
B2	0.05	45	2	45	0	0.00
B3	0.09	270	1	180	0	0.00
B4	0.19	180	0	-	0	-
B5	0.25	90	2	-	0	0.00
B6	0.09	v	0	-	0	-
B7	0.08	270	0	-	0	-
B8	0.10	v	0	-	0	-
B9	0.08	v	3	-	3	1.00
B10	0.03	180	18	90	17	0.94
B11	0.03	180	2	90	0	0.00
C1	0.23	45	1	45	0	0.00
C2	0.07	90	0	-	0	-
C3	0.20	180	1	90	0	0.00
C4	0.30	v	0	-	0	-
C5	0.28	v	0	-	0	-
C6	0.14	225	0	-	0	-
C7	0.11	180	0	-	0	-
C8	0.13	180	0	-	0	-
C9	0.08	180	4	180	3	0.75
C10	0.04	135	0	-	0	-
C11	0.05	180	0	-	0	-
D1	0.06	0	0	-	0	-
D2	0.06	180	0	-	0	-
D3	0.13	135	0	-	0	-
D4	0.34	180	0	-	0	-
D5	0.23	180	0	-	0	-
D6	0.17	180	0	-	0	-
D7	0.10	180	0	-	0	-
D8	0.14	180	0	-	0	-
D9	0.08	135	0	-	0	-
D10	0.11	135	0	-	0	-
D11	0.03	135	1	135	0	0.00

Table 7.2: continued

grid position	flow velocity (cm s^{-1})	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
E1	0.09	315	0	-	0	-
E2	0.06	v	0	-	0	-
E3	0.14	180	0	-	0	-
E4	0.19	180	0	-	0	-
E5	0.30	180	0	-	0	-
E6	0.17	180	0	-	0	-
E7	0.13	90	0	-	0	-
E8	0.07	v	0	-	0	-
E9	0.14	135	1	135	1	1.00
E10	0.14	90	0	-	0	-
E11	0.04	135	0	-	0	-
F1	0.14	315	1	45	0	0.00
F2	0.06	45	0	-	0	-
F3	0.05	135	0	-	0	-
F4	0.23	225	0	-	0	-
F5	0.18	180	0	-	0	-
F6	0.21	v	0	-	0	-
F7	0.25	0	1	0	1	1.00
F8	0.25	0	0	-	0	-
F9	0.14	45	0	-	0	-
F10	0.06	90	0	-	0	-
F11	0.03	90	0	-	0	-
G1	0.09	315	0	-	0	-
G2	0.07	90	2	90	2	1.00
G3	0.13	180	8	-	8	1.00
G4	0.25	180	3	180	2	0.67
G5	0.27	180	47	90	47	1.00
G6	0.17	v	6	-	4	0.67
G7	0.13	v	6	-	4	0.67
G8	0.14	v	7	-	5	0.71
G9	0.11	45	9	45	8	0.89
G10	0.03	135	2	135	0	0.00
G11	0.05	180	1	90	0	0.00

v = variable

Table 7.3: Flow velocity and fish behaviour results - 1.0 l min^{-1}

grid position	flow velocity ($cm\ s^{-1}$)	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
A1	0.18	90	5	-	4	0.80
A2	0.16	0	47	90	30	0.64
A3	0.24	270	19	0	16	0.84
A4	0.21	180	8	90	4	0.50
A5	0.40	270	3	0	1	0.33
A6	0.26	225	15	45	14	0.93
A7	0.34	225	1	45	0	0.00
A8	0.16	315	1	135	1	1.00
A9	0.26	315	1	135	0	0.00
A10	0.08	315	3	45	0	0.00
A11	0.06	225	2	45	1	0.50
B1	0.58	0	4	180	3	0.75
B2	0.29	135	2	45	2	1.00
B3	0.09	180	3	-	3	1.00
B4	0.63	180	0	-	0	-
B5	0.32	180	0	-	0	-
B6	0.34	270	0	-	0	-
B7	0.42	315	1	135	1	1.00
B8	0.58	315	0	-	0	-
B9	0.37	270	0	-	0	-
B10	0.23	270	0	-	0	-
B11	0.10	270	0	-	0	-
C1	0.37	90	26	90	26	1.00
C2	0.40	180	0	-	0	-
C3	0.44	180	2	90	0	0.00
C4	0.47	135	0	-	0	-
C5	0.26	180	0	-	0	-
C6	0.44	180	0	-	0	-
C7	0.23	225	0	-	0	-
C8	0.32	225	1	45	1	1.00
C9	0.42	225	0	-	0	-
C10	0.12	270	0	-	0	-
C11	0.12	180	0	-	0	-
D1	0.21	0	2	180	2	1.00
D2	0.26	225	0	-	0	-
D3	0.83	180	0	-	0	-
D4	0.47	180	0	-	0	-
D5	0.63	180	0	-	0	-
D6	0.40	180	0	-	0	-
D7	0.47	180	0	-	0	-
D8	0.42	180	0	-	0	-
D9	0.50	180	0	-	0	-
D10	0.34	180	0	-	0	-
D11	0.10	180	1	90	0	0.00

Table 7.3: continued

grid position	flow velocity (cm s^{-1})	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
E1	0.63	315	0	-	0	-
E2	0.36	225	0	-	0	-
E3	0.53	180	2	180	2	1.00
E4	0.68	180	0	-	0	-
E5	0.53	180	0	-	0	-
E6	0.34	180	1	180	1	1.00
E7	0.16	180	0	-	0	-
E8	0.53	180	0	-	0	-
E9	0.34	180	0	-	0	-
E10	0.30	180	0	-	0	-
E11	0.16	v	2	-	2	1.00
F1	0.67	270	0	-	0	-
F2	0.16	270	3	90	3	1.00
F3	0.30	135	0	-	0	-
F4	0.25	v	1	-	0	0.00
F5	0.31	135	0	-	0	-
F6	0.27	90	35	0	35	1.00
F7	0.50	90	0	-	0	-
F8	0.32	90	0	-	0	-
F9	0.24	45	0	-	0	-
F10	0.42	45	0	-	0	-
F11	0.10	45	0	-	0	-
G1	0.47	0	1	0	1	1.00
G2	0.47	45	6	45	4	0.67
G3	0.15	90	11	0	10	0.91
G4	0.08	180	5	180	3	0.60
G5	0.21	0	13	0	12	0.92
G6	0.58	135	42	45	42	1.00
G7	0.30	90	8	90	4	0.50
G8	0.40	45	12	45	8	0.67
G9	0.15	45	6	45	2	0.33
G10	0.16	v	3	-	0	0.00
G11	0.06	225	2	135	0	0.00

v = variable

Table 7.4: Flow velocity results - 2.0 and 4.0 l min^{-1}

grid position	2.0 l min^{-1} flow velocity (cm s^{-1})	2.0 l min^{-1} flow dir. (deg.)	4.0 l min^{-1} flow velocity (cm s^{-1})	4.0 l min^{-1} flow dir. (deg.)
A1	1.09	45	1.92	45
A2	0.93	225	1.70	v
A3	0.47	v	1.92	90
A4	1.09	180	1.25	v
A5	1.09	135	2.50	90
A6	0.93	0	1.39	0
A7	0.68	0	1.92	0
A8	0.83	0	1.39	315
A9	0.76	270	1.39	225
A10	0.53	v	1.25	315
A11	0.20	0	1.25	225
B1	1.25	90	2.50	45
B2	0.58	225	0.83	180
B3	0.83	180	1.25	v
B4	1.09	180	1.47	45
B5	0.83	v	2.50	v
B6	0.83	315	0.83	270
B7	0.93	315	1.47	v
B8	1.09	315	1.47	v
B9	0.63	270	0.76	270
B10	0.29	270	1.47	270
B11	0.19	315	0.26	315
C1	1.25	45	2.50	90
C2	0.76	135	1.92	135
C3	1.25	180	1.92	135
C4	1.92	225	1.92	180
C5	1.25	225	2.50	225
C6	1.25	180	1.39	225
C7	1.09	180	2.50	225
C8	0.93	225	2.50	180
C9	0.58	225	1.39	270
C10	0.42	v	1.39	180
C11	0.17	135	0.32	180
D1	0.20	45	1.39	v
D2	1.09	180	2.50	180
D3	1.25	180	2.50	180
D4	1.47	180	2.50	180
D5	1.25	180	2.50	180
D6	1.25	180	2.50	180
D7	1.47	180	2.50	225
D8	1.25	180	2.50	225
D9	0.93	135	1.25	180
D10	0.63	180	1.47	180
D11	0.47	180	1.09	180

Table 7.4: continued

grid position	2.0 l min^{-1} flow velocity (cm s^{-1})	2.0 l min^{-1} flow dir. (deg.)	4.0 l min^{-1} flow velocity (cm s^{-1})	4.0 l min^{-1} flow dir. (deg.)
E1	1.09	270	2.50	270
E2	0.83	180	1.92	180
E3	0.68	180	2.50	180
E4	0.50	180	2.50	180
E5	1.09	180	2.50	180
E6	0.93	135	1.92	180
E7	0.58	180	1.09	180
E8	1.09	180	1.25	180
E9	0.68	135	1.25	135
E10	0.76	135	1.47	135
E11	0.93	135	1.92	135
F1	1.47	315	2.50	315
F2	0.37	180	1.09	225
F3	0.68	v	1.92	180
F4	0.93	180	1.92	225
F5	1.25	v	1.92	180
F6	0.76	90	1.92	90
F7	0.93	45	1.25	45
F8	1.09	90	1.92	45
F9	0.93	v	1.25	90
F10	0.58	180	1.92	45
F11	0.63	135	1.25	135
G1	0.63	v	1.92	0
G2	0.68	v	2.50	0
G3	0.76	v	1.47	90
G4	0.53	135	1.92	180
G5	0.83	180	0.83	225
G6	0.83	90	0.83	v
G7	1.47	v	1.92	45
G8	1.47	90	2.50	45
G9	0.50	90	1.47	0
G10	0.83	135	1.92	45
G11	0.39	135	0.47	135

v = variable

Table 7.5: Flow velocity and fish behaviour results - 8.0 l min^{-1}

grid position	flow velocity ($cm\ s^{-1}$)	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
A1	6.69	225	17	45	16	0.94
A2	4.70	180	55	90	36	0.65
A3	3.10	180	40	90	31	0.78
A4	4.88	135	18	45	10	0.56
A5	7.37	135	9	135	2	0.22
A6	7.48	45	6	45	5	0.83
A7	5.70	0	9	90	7	0.78
A8	5.40	0	13	90	10	0.77
A9	5.00	0	26	90	22	0.85
A10	4.20	0	3	90	0	0.00
A11	2.50	v	0	-	0	-
B1	5.20	270	15	90	14	0.93
B2	3.00	270	7	180	6	0.86
B3	4.50	180	0	-	0	-
B4	6.01	135	0	-	0	-
B5	4.20	180	0	-	0	-
B6	3.30	270	0	-	0	-
B7	6.10	315	0	-	0	-
B8	5.22	315	0	-	0	-
B9	3.50	270	0	-	0	-
B10	5.42	315	0	-	0	-
B11	0.00	v	2	-	1	0.50
C1	6.84	135	2	45	1	0.50
C2	6.40	180	1	0	1	1.00
C3	7.10	180	0	-	0	-
C4	3.50	180	0	-	0	-
C5	4.72	225	0	-	0	-
C6	5.06	225	0	-	0	-
C7	5.37	225	0	-	0	-
C8	5.17	225	0	-	0	-
C9	4.18	225	0	-	0	-
C10	0.00	v	0	-	0	-
C11	0.00	v	0	-	0	-
D1	3.92	v	0	-	0	-
D2	8.40	180	1	180	0	0.00
D3	10.00	180	0	-	0	-
D4	10.00	180	0	-	0	-
D5	9.60	180	0	-	0	-
D6	7.70	180	0	-	0	-
D7	8.00	180	0	-	0	-
D8	6.80	180	0	-	0	-
D9	6.00	180	0	-	0	-
D10	3.70	180	0	-	0	-
D11	3.50	90	0	-	0	-

Table 7.5: continued

grid position	flow velocity (cm s^{-1})	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
E1	6.02	225	0	-	0	-
E2	8.30	180	0	-	0	-
E3	9.30	180	0	-	0	-
E4	9.50	180	0	-	0	-
E5	6.50	180	0	-	0	-
E6	5.20	180	0	-	0	-
E7	4.20	180	2	180	2	1.00
E8	2.80	180	0	-	0	-
E9	3.92	v	0	-	0	-
E10	4.20	90	0	-	0	-
E11	0.00	v	0	-	0	-
F1	6.44	225	0	-	0	-
F2	4.84	135	2	135	1	0.50
F3	4.90	180	0	-	0	-
F4	6.20	180	0	-	0	-
F5	4.67	135	0	-	0	-
F6	3.50	90	0	-	0	-
F7	6.38	v	3	-	3	1.00
F8	6.27	45	3	135	3	1.00
F9	6.51	45	1	135	1	1.00
F10	5.09	45	0	-	0	-
F11	4.10	135	2	135	0	0.00
G1	5.70	v	0	-	0	-
G2	4.00	90	1	90	0	0.00
G3	4.08	135	16	135	14	0.88
G4	6.04	135	4	135	2	0.50
G5	5.40	180	2	0	1	0.50
G6	6.00	180	3	180	2	0.67
G7	7.29	v	23	-	22	0.96
G8	6.50	180	3	0	3	1.00
G9	3.80	0	10	0	7	0.70
G10	4.50	0	0	-	0	-
G11	0.00	v	1	-	0	0.00

v = variable

Table 7.6: Flow velocity and fish behaviour results - 13.0 l min^{-1}

grid position	flow velocity (cm s^{-1})	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	(prop)
A1	6.23	45	0	-	0	-
A2	4.00	270	1	0	0	0.00
A3	3.70	180	0	-	0	-
A4	5.20	180	2	-	0	0.00
A5	4.90	180	0	-	0	-
A6	7.14	v	2	-	0	0.00
A7	4.20	0	0	-	0	-
A8	4.30	0	2	90	1	0.50
A9	4.90	315	5	45	3	0.60
A10	3.00	315	0	-	0	-
A11	0.00	225	0	-	0	-
B1	5.20	0	0	-	0	-
B2	5.77	225	9	135	6	0.67
B3	4.20	180	10	90	7	0.70
B4	6.00	0	5	90	3	0.60
B5	7.30	0	0	-	0	-
B6	6.40	0	1	90	1	1.00
B7	7.72	315	2	135	2	1.00
B8	6.92	315	3	45	2	0.67
B9	5.67	315	0	-	0	-
B10	4.20	270	1	180	0	0.00
B11	0.00	225	0	-	0	-
C1	6.70	90	0	-	0	-
C2	7.30	180	5	90	2	0.40
C3	14.70	180	10	90	9	0.90
C4	10.80	180	0	-	0	-
C5	7.70	225	8	135	7	0.88
C6	6.20	180	0	-	0	-
C7	7.50	180	2	-	2	1.00
C8	5.99	225	0	-	0	-
C9	4.20	270	0	-	0	-
C10	4.68	225	3	-	0	0.00
C11	3.75	225	0	-	0	-
D1	5.70	135	0	-	0	-
D2	11.70	180	10	90	6	0.60
D3	13.00	180	12	90	7	0.58
D4	12.20	180	3	90	2	0.67
D5	9.90	180	2	-	0	0.00
D6	9.50	180	1	90	1	1.00
D7	9.10	225	3	45	2	0.67
D8	6.80	180	1	90	1	1.00
D9	5.40	180	0	-	0	-
D10	5.88	135	0	-	0	-
D11	5.22	135	1	135	0	0.00

Table 7.6: continued

grid position	flow velocity (cm s^{-1})	flow dir. (deg.)	fish occurrence (no.)	direction diff. (deg.)	fish settled (no.)	fish settled (prop)
E1	8.27	225	0	-	0	-
E2	9.80	180	22	90	15	0.68
E3	10.30	180	16	90	9	0.56
E4	8.70	180	5	90	4	0.80
E5	7.10	180	4	90	1	0.25
E6	5.80	180	4	90	0	0.00
E7	6.84	135	2	-	2	1.00
E8	5.89	v	1	-	0	0.00
E9	6.98	135	1	135	0	0.00
E10	4.30	90	0	-	0	-
E11	7.79	135	0	-	0	-
F1	9.20	270	3	90	1	0.33
F2	8.94	135	27	45	7	0.26
F3	7.70	180	22	90	17	0.77
F4	7.70	180	1	90	0	0.00
F5	9.30	v	3	-	1	0.33
F6	8.71	45	5	45	3	0.60
F7	6.75	45	6	135	4	0.6
F8	7.29	45	3	135	1	0.33
F9	5.00	90	1	90	1	1.00
F10	6.40	90	1	0	0	0.00
F11	4.50	90	2	-	1	0.50
G1	8.14	315	1	135	0	0.00
G2	8.63	135	13	135	0	0.00
G3	7.50	180	17	180	4	0.24
G4	7.10	180	13	-	1	0.08
G5	8.06	v	1	-	0	0.00
G6	7.82	45	5	45	1	0.20
G7	7.55	45	4	45	0	0.00
G8	7.74	45	4	45	1	0.25
G9	3.50	90	8	90	3	0.38
G10	5.66	45	0	-	0	-
G11	4.48	135	1	45	0	0.00

v = variable

influent end.

With the exception of fish in the 13 l min^{-1} tank, the distribution of fish within the treatment tanks was similar. On the majority of occasions the fish were positioned either in one of the corners of the tank or at the centre of the side walls. These positions were similar to those described as having the greatest variation in flow direction. In the 13 l min^{-1} tank, the fish were often located in front of the inlet flow pipe across the influent end of the tank, though fish were also more dispersed throughout the tank than in other treatments.

Statistical analysis of flow direction, flow velocity, fish location, orientation and movement measurements was conducted, as described in section 7.3.6, to quantify any influences of the physical parameters measured, on behavioural aspects. Table 7.7 summarizes the results of these analyses. No significant correlations between the parameters tested occurred at 0.5 l min^{-1} and no non-significant associations were evident.

In the tank with a flow rate of 1.0 l min^{-1}, the total number of times fish occurred in and settled on each square, decreased significantly with increased flow direction, though plots of the association suggested that there was a reduction in the greatest number of times fish

Table 7.7 Summary of flow / behaviour correlation analyses results

Y parameter	X parameter	correlation coefficient r	sample size n	sign. level NS / p<
0.5 l min^{-1}				
flow dir.	flow vel.	-0.033	66	NS
dir. diff.	flow vel.	-0.103	27	NS
fish no.	flow vel.	0.132	77	NS
settled no.	flow vel.	0.145	77	NS
settled prop.	flow vel.	0.121	35	NS
fish no.	flow dir.	0.048	66	NS
settled no.	flow dir.	0.012	66	NS
settled prop.	flow dir.	-0.132	31	NS
1.0 l min^{-1}				
flow dir.	flow vel.	0.002	74	NS
dir. diff.	flow vel.	0.035	32	NS
fish no.	flow vel.	-0.095	77	NS
settled no.	flow vel.	-0.040	77	NS
settled prop.	flow vel.	0.249	37	NS
fish no.	flow dir.	-0.330	74	**0.01**
settled no.	flow dir.	-0.302	74	**0.01**
settled prop.	flow dir.	-0.274	34	NS
8.0 l min^{-1}				
flow dir.	flow vel.	0.060	66	NS
dir. diff.	flow vel.	-0.067	27	NS
fish no.	flow vel.	-0.039	77	NS
settled no.	flow vel.	-0.027	77	NS
settled prop.	flow vel.	0.261	31	NS
fish no.	flow dir.	-0.143	66	NS
settled no.	flow dir.	-0.128	66	NS
settled prop.	flow dir.	0.110	27	NS
13.0 l min^{-1}				
flow dir.	flow vel.	-0.007	73	NS
dir. diff.	flow vel.	0.007	43	NS
fish no.	flow vel.	0.407	77	**0.001**
settled no.	flow vel.	0.415	77	**0.001**
settled prop.	flow vel.	0.247	54	NS
fish no.	flow dir.	0.026	73	NS
settled no.	flow dir.	0.074	73	NS
settled prop.	flow dir.	-0.002	50	NS
Combined data				
flow dir.	flow vel.	-0.031	279	NS
dir. diff.	flow vel.	0.066	131	NS
fish no.	flow vel.	0.041	308	NS
settled no.	flow vel.	-0.022	308	NS
settled prop.	flow vel.	-0.066	156	NS
fish no.	flow dir.	-0.104	279	NS
settled no.	flow dir.	-0.095	279	NS
settled prop.	flow dir.	-0.112	141	NS

were present or settled per square, with increased direction degrees.

There were no significant correlations or obvious patterns in the results of the 8.0 l min^{-1} flow rate treatment. At the greatest inflow rate tested (13.0 l min^{-1}), the number of times fish were present in and settled on each square was significantly correlated with the local flow velocity. Fish were more often present at and settled in grid squares with a higher flow velocity, though this association was not apparent in the combined data (Table 7.7).

Multiple regression analysis results indicated that, at 1.0 and 13.0 l min^{-1}, the number of times fish were present at, and settled on, a square was related to both the flow velocity and flow direction at the square (Table 7.8). The four equations describing the associations however accounted for less than 20 % of the variability of the data each. The significance of the overall equation results from the association of one of the two parameters in each case.

Table 7.9 indicates the number of times fish were swimming and settled at different flow rates and tank mean flow velocities. The number of occasions fish were swimming was not significantly correlated with either flow rate or mean flow velocity ($r=-0.913$, $df=2$, $p>0.05$ and $r=-0.835$, $df=2$, $p>0.05$ respectively), though the

Table 7.8: Summary of flow / behaviour multiple regression results

x parameters are flow velocity and flow direction:

flow rate	y parameter	sample size n	prob. level p<	fitted equation	explained variability %
0.5	fish no.	66	0.422		
0.5	settled no.	66	0.397		
0.5	settled prop.	31	0.638		
1.0	fish no.	74	**0.011**	no=11.9-5.40vel -0.035dir	11.9
1.0	settled no.	74	**0.031**	no=8.86-2.34vel -0.028di	9.3
1.0	settled prop.	34	0.156		
8.0	fish no.	66	0.176		
8.0	settled no.	66	0.188		
8.0	settled prop	27	0.864		
13.0	fish no.	73	**0.001**	no=2.59+0.94vel +0.002dir	17.5
13.0	settled no.	73	**0.001**	no=2.30+0.56vel +0.003dir	19.0
13.0	settled prop.	50	0.203		
all	fish no.	279	0.208		
all	settled no.	279	0.215		
all	settled prop.	156	0.301		

correlation between the number of swimming fish and flow rate was the greater. Figures 7.9 and 7.10 however indicated a pronounced decrease in the number of fish settled with increased flow rate and with increased tank mean flow velocity. A chi-squared analysis of this data indicated that the null hypothesis, "that the proportions of settled and swimming fish were the same at each treatment", could be rejected (chi^2=112.48, df=3, p>0.001).

Table 7.9: Fish movement at different flow velocities and flow rates

Flow rate (l min^{-1})	Mean flow velocity (cm s^{-1})	occasions settled (no.)	occasions swimming (no.)
0.5	0.13	246	54
1.0	0.33	238	62
8.0	5.22	223	77
13.0	6.76	141	159

7.5 DISCUSSION

Despite large differences in flow rate, relative to tank volume, flow velocity profiles near the bottom of the tank, where turbot live, were similar during different treatments. Flow visualization studies conducted at a range of depths, with similar flow rates, indicated marked differences in flow patterns in the treatment tanks. Alternatively, results of tracer and flow meter quantifications of flow patterns in a tank with a range of flow rates and the same depth, described in this chapter, indicated a similar pattern of flows. Comparing results

Figure 7.9: Changes in the number of times fish settled with increased flow rates (0.5 - 13.0 l/min)

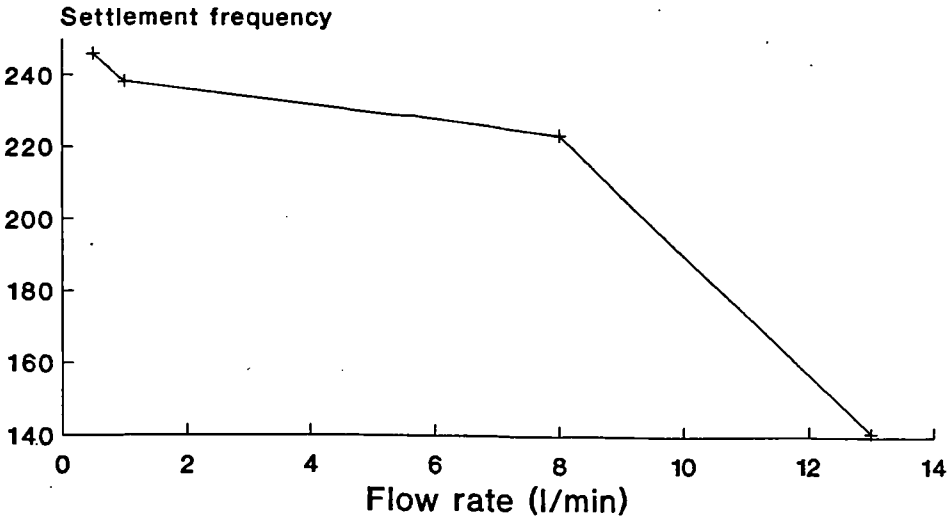
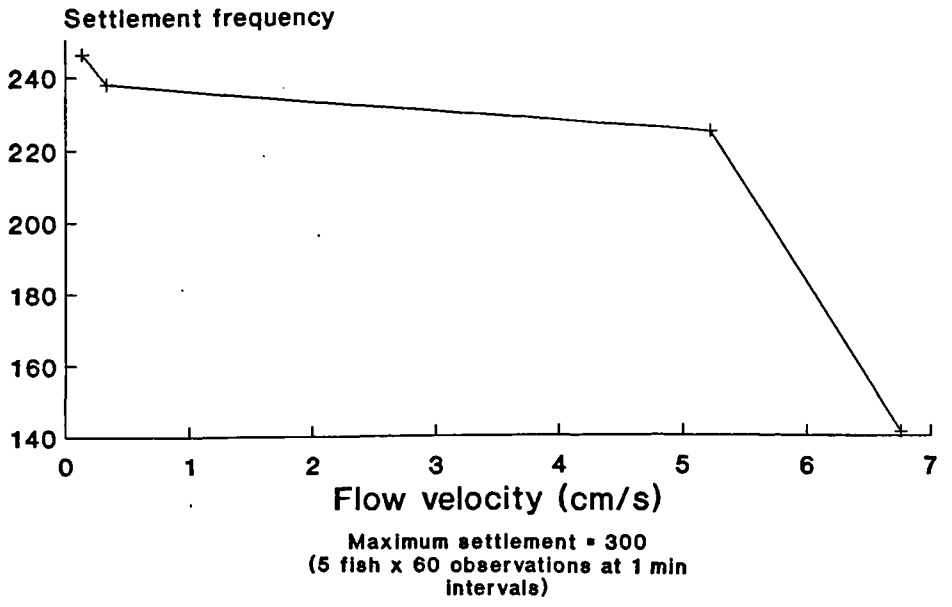


Figure 7.10: Changes in the number of times fish settled with increased tank mean velocity (at 1 cm above tank floor)



described in this chapter with results described in Chapter 6, it would appear that depth was a more important determinant of local flow velocities near the tank floor, than flow rate, within the range of depths and flow rates studied.

In shallow tanks flow visualization results indicated that there was a high degree of mixing, caused primarily by surface and boundary layer drag. In the deepest tank a more plug flow of water from the inlet to outlet was observed but, because the outlet design was inadequate to remove all the plug, reflection off the effluent end wall back along the tank to the influent end wall, in the opposite direction to the initial flow, was observed. Mixing of the reflected influent water with the rest of the tank water, was caused by boundary layer drag and shear stress at the boundary of the two flows in opposite directions. The 9 cm deep tank was intermediate between these situations.

Results from tracer and flowmeter tests further clarified the situation. The initial flow from inlet to outlet, described above, must have occurred near the surface, because flow velocities near the floor of the tank were in the opposite direction. At the effluent end of the tank the plug flow was diverted downward towards the tank floor and then back towards the influent end of the tank. This is indicated by slow horizontal water flow velocities and variable flow directions at the effluent

end wall. The reflected flow proceeded back towards the inflow end of the tank near the tank floor, as indicated by flow velocities in this direction. Laminar flows occurred near the centre of the tank away from the side walls. Boundary layer frictional drag was considered an important aspect determining flow distribution (section 6.6) and this is confirmed by the non-laminar flows at the side walls. Flows, generally towards the centre of the tank, at the side walls were probably caused by down eddies from the overlying initial flow from the influent pipe. At the inflow end of the tank further mixing and upwelling is indicated by the variable flow directions.

A combination of flow quantification and visualization studies were therefore successful in describing flows and mixing within the tanks.

The generally similar distribution of fish settlement in the treatment tank with different flow rates was probably due to two causes. Firstly an external environmental parameter such as light may have influenced fish position preference. Most fish occurrences were near to the tank walls, so the fish may have been using the walls for shelter, in the absence of a particulate substrate in which to bury. If this were the case it is expected that the fish would have preferred to settle in the tank corners which would have offered more shelter than straight walls. Settlement in more than one corner was not however noticed. It is possible that the transfer

of fish from tanks with a stocking density of 30 fish $tank^{-1}$ to a tank with only one occupant may have increased any stress reaction to external parameters. Honer et al. (1987b) showed that in higher stocking densities of juvenile tilapia (Sarotherodon galilaeus) the irritation and fright reaction after external disturbance was reduced, and attributed this to a reduction in the distance between individuals This possibility was considered preferable to the effect that interaction between fish in a tank may have had on fish movement, orientation and location. The effects of behavioural interaction would have been highly variable and difficult to define, whereas the stress due to solitary confinement was constant at all treatments. An extension of the discussion by Honer et al. (1987b) suggests that the effect on behaviour which an environmental parameter such as flow rate exerted, may be increased by a reduction in stocking density, which would be advantageous in this study in indicating such effects. Results of this study may then not be applicable to high stocking density commercial culture situations.

A second explanation for fish settlement location was that the turbot chose areas of turbulence. The greatest area of turbulence at the tank floor, was near to the effluent end wall. Fish did not however tend to settle in this region. Other areas of turbulence, as indicated by vertical eddies, expected to be of less strength than at the effluent end wall, have been shown to occur along the

side and influent end walls, and it was there that the majority of fish settlements occurred. The boundary layer effect may explain this phenomenon. Flow velocity decreases with decreased distance from a boundary (Burrows and Chenoweth, 1955) such as a tank wall or floor. Turbot, as a demersal fish, predominantly lie on the substrate. The fish will therefore live in a range of local flow velocities which will depend on the vertical height of the fish and the overlying flow velocity. The ventral side of the fish not resting on the floor will be in a very low flow velocity, while the dorsal surface, depending on the size of the fish, may lie in the path of more laminar, faster flow. Depending on the width of the boundary layer the turbot may lie in a microclimate of slow moving or even dead water, which is very different to the rest of the tank. Vertical eddies will reduce this boundary layer by introducing fluid with a momentum perpendicular to the tank floor. A consequence of this effect would be a reduction in the width of the dead layer with a subsequent improvement in water quality at the tank floor, assuming that water quality of the overlying layers of water was superior. The situation is further complicated by the presence of the fish itself which will both act as a boundary and an obstacle to flow, producing its own boundary layer and eddies respectively. The pattern of fish distribution with respect to the defined flows may then have been due to positioning of the fish in vertical eddies which disrupt the boundary layer, but not in flows so great that energy would have been required to

maintain position. This latter suggestion has not been tested in this study, but the influence of flow velocity on fish position will be discussed below.

The preference of the fish for a position below the influent spray pipe in the 13 l min^{-1} treatment tank does not, as might initially be expected, completely disagree with the previous hypothesis. Flow velocities at this flow rate were higher than in other treatments. Down eddies may then have been stronger, at this high inflow rate, in the locations the fish settled in other treatments. The strong bottom currents in the region in front of the inflow spray pipe, generated by the influent water momentum, would be expected to reduce the boundary layer depth. However previous suggestions, which indicated that regions of flows which may require effort to maintain position are avoided, are not confirmed by this fish distribution. Casual observations of fish behaviour at this treatment indicated a behaviour pattern unique within the treatments tested. The fish lay on the tank floor below the influent stream and periodically swam vigorously into the stream for short periods. It is possible that at these higher flow rates different behavioural responses to flow, rather than physical differences due to the flow, may have resulted in the observed settlements.

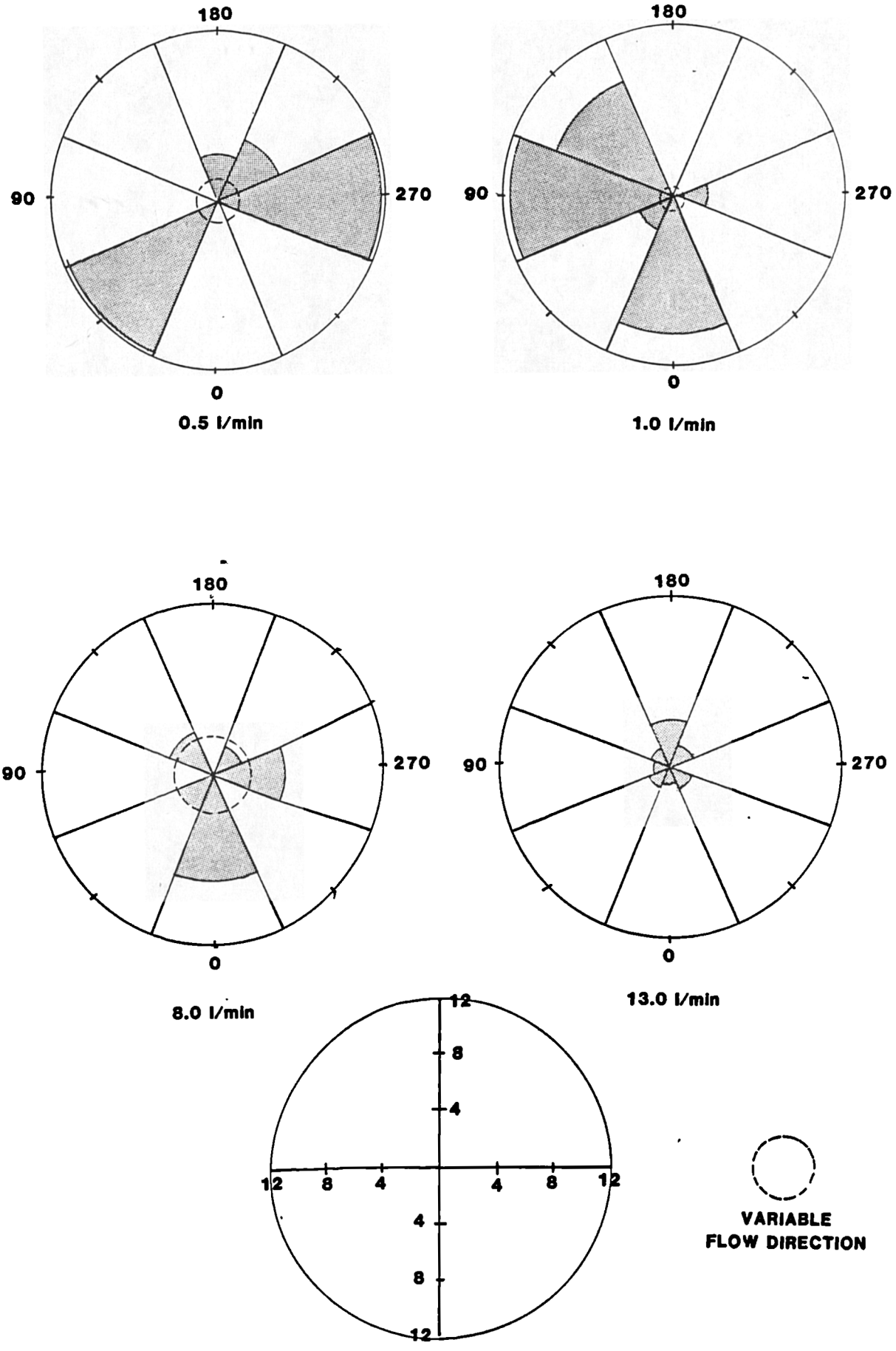
Further examination of the influence of flow velocity in the vicinity of the fish, on position of the fish and

their movement, indicated that at all flow rates other than 13.0 l min^{-1}, there were no associations. The difference in fish behaviour at this treatment, compared with other treatments, supports the suggestion that other factors were responsible at the higher flow rate. At this treatment the fish were more often found at, or settled on, areas of the tank with the greatest flow velocity, thus confirming distribution statements based on Figure 7.6. Overall no preference for high or low flow velocity was evident. Flow velocities in this study were measured in the horizontal plane: no measurement was made of vertical flow velocities. While avoidance, by the turbot, of high flow velocity areas in 0.5 - 8.0 l min^{-1} treatment tanks was not statistically proved, location preference due to vertical flows also cannot be discounted. Preference for higher flow velocities in tanks with generally higher flow velocities throughout is confirmed.

The reasons for a significant association between flow direction and the number of occurrences of fish, and between flow direction and the number of times fish settled, are unclear. For the purposes of measurement of flow direction there were two extremes of flow direction: 0 and 315 ^{o}. In practice these two directions were adjacent, so a more synodal pattern of association rather than linear was expected. If the parameters are actually associated, the number of occasions in which a fish was seen to orientate in a particular direction in a particular square, increased clockwise from 0 - 315 ^{o}.

Care should be taken in interpreting this result. The number of squares in which different flow directions occurred was variable. Settlement and occurrence values were therefore calculated as a mean of positions with the same flow direction, not as a mean of all positions in the tank. So the number of times a square was selected to be settled on, or be present in, decreased with increased direction degrees. Regions with a flow perpendicular to the long axis of the tank (ie. 90 °) tended to be areas of greater mixing and vertical eddies, so this result confirms suggestions, explaining the position preference of the fish, described above. Statistical analysis of the total number of times fish settled on, or were present in, all squares of the same flow direction was not possible, because the number of squares with each flow direction was not the same, so the resulting frequency of occurrences did not have the same opportunity to occur, hence analysis was conducted as described above. Figure 7.11 illustrates the significant result from the 1.0 l min^{-1} treatment and shows the flow direction preference at other flow rates. A clear pattern is not evident, but the number of settlements appeared to decrease with increased flow rate. This would be expected if the fish were swept by the flow off their preferred position or they were attempting to find a suitable position to settle. The chi-squared analysis of settled and swimming fish did not however confirm this suggestion. Results illustrated in Figure 7.11 have been compiled from unequal numbers of grid

Figure 7.11: Mean number of fish settlements per grid square at a range of flow rates (9 cm water depth)



squares, so it is possible that fish may not have located a less frequently occurring flow direction, resulting in low mean settlement levels.

Although multiple regression analysis results did indicate that the gradient of the line of best fit describing the influence of both flow velocity and flow direction on the number of fish present and on the number of settled fish, was significantly different from zero, the equation describing the line accounted for only a small proportion of the variability. This meant that whilst an association between the variables could be determined, the equation describing the line was not useful for predictive statistics. In summary, both flow velocity and flow direction could not be considered to act together to influence the behaviour of the turbot in this study.

A combination of data from different flow rate treatments did not indicate any significant influence of the tank hydrodynamics on fish behaviour. The summary data (Table 7.9) did however indicate that fish swam more in tanks with either a faster flow rate or faster mean velocity of flows near the floor of the tank. Reasons for this have been suggested above. Several consequences of this situation are evident. In higher flow rate tanks, fish will be expected to expend more energy swimming. This may cause lower growth rates, higher food conversion ratios and lower condition factors because of the

greater energy used or because of stress, as has been shown by many workers including Dave et al. (1975). Feeding behaviour may also be adversely affected if the fish are swimming more frequently, with similar consequences. In the faster flow rate tanks however, water quality may be better, as suggested by Poxton and Allouse (1987), producing improved growth rates, food conversion ratios and survival. The link between water quality and specific growth rate has been suggested by Rosenthal et al. (1982). Further, an increase in swimming activity may produce a better quality of flesh, which occurs in salmonids (Burrows and Combes, 1968). This may yield a higher market price and improve the physical fitness of the fish. Such aspects were not considered in this study and so have not been verified. If the detrimental aspects of increased swimming can be offset by the beneficial aspects economically, then increased flow rates may be advantageous. Another important aspect to consider if flow rates may be increased, is the increased pumping costs and water requirements which will be needed. This chapter is concerned with the effects of flow velocity on fish behaviour, so a full discussion of results presented here in relation to other criteria covered in other chapters will be discussed in Chapter 11.

7.6 CONCLUSIONS

1. Water depth appeared a more important determinant of flow velocity patterns in the treatment tank, than flow rate, within the ranges tested in this study.
2. A pronounced surface flow from the inlet was reflected off the effluent end wall and side walls, producing regions of vertical eddies and a bottom flow in the opposite direction to that at the surface.
3. In the 0.5 - 8.0 l min^{-1} treatments, fish appeared to position themselves in these vertical eddy regions.
4. Preference of location in the tank in relation to flow velocity or flow direction was demonstrated on two occasions (13.0 and 1.0 l min^{-1} treatments respectively): combining data from all treatments, no significant preference was evident.
5. Swimming activity increased significantly, and therefore the settlement rate decreased, with increased flow rate and with increased mean flow velocity near the floor of the tank.

CHAPTER 8

WATER DEPTH EXPERIMENT 1

CONSTANT STOCKING DENSITY BY AREA

8.1 INTRODUCTION

The literature review (Chapter 3) indicated that almost all aquaculture tanks to date have been designed for pelagic species such as salmonids. Few tanks have either been designed or even specifically managed to hold benthic or demersal species. Many cultured animals fall into this category and include: molluscs eg. Crassostrea gigas; crustaceans eg. Penaeus japonica, Macrobrachium rosenbergii and Homarus gammarus; and fish eg. Scophthalmus maximus and Hippoglossus hippoglossus. Such species spend the majority of their juvenile and adult lives on the substrate. They can be thought of as requiring space principally in terms of surface area rather than volume.

In a deep tank, relative to the size of the animals, a large volume of mid- and surface water is unoccupied. Whether this can be considered to be wasted may depend on the hydrodynamics of the tank, ie. the extent of mixing. A tank design, or adaptation to an existing design, specific to demersal animals therefore may provide only a shallow water depth, thus increasing the floor area to volume ratio.

Disadvantages associated with the current system of relatively deep tanks include the possibility of poor hydrodynamics and a reduction in laminar flow. Burley and Klapsis (1985) deduced that the velocity of water which entered a tank was dissipated more by the greater volume of a deeper tank, which resulted in an increased "dead volume". This probably adversely affected water quality. Regions of poor mixing, "dead volumes", which contain water of a low DO and high nitrogenous metabolite concentration, may build up. Rosenthal et al. (1982; 1985) proved a link between dead areas and poor water quality by showing that dangerously high levels of unionized ammonia and low DO concentrations occurred at certain times of the day in circular tanks with these dead areas.

Possible advantages of a deep tank include a large unoccupied volume of water which may act as a sink for wastes and a store of nutrients, supplying deeper levels and buffering fluctuations in water quality. Vertical movement of the animals will be less restricted.

If a shallow depth is maintained, the volume will be reduced and water flow may be more laminar, though Burrows and Chenoweth (1955) suggested that shallower ponds had a less uniform velocity distribution, due to boundary layer effects. Reduction in depth may have several consequences. Residence time will be less than in a deeper tank, of the same design, at the same flow rate.

Water will therefore either pass through the tank quicker, resulting in an increased flushing through of water, at no increase in flow rate, or to obtain the same residence time, flow rate would be reduced in shallower tanks. A reduced flow rate would result in savings in pumping costs and water conservation in situations where water may be scarce.

Alternatively a reduction in depth within a tank without altering the biomass may have many detrimental effects. The build up of nitrogenous metabolites will be quicker because of the greater stocking density per unit volume. Honer et al. (1987a) indicated that an increase in the amplitude of diurnal fluctuations in, and overall concentration of, total ammonia levels occurred with an increased stocking density in 42 l aquaria. A shorter residence time may therefore be required to maintain adequate water quality, so savings in the quantity of water used, by reducing flow rate, may not be possible. Honer et al. (1987a) showed a small decrease in the diurnal peak of total ammonia levels with increased flow rate, though the maximum flow rate was only 2 l min^{-1}. Rosenthal et al. (1984) also showed that ammonia fluctuations could be influenced by changes in the hydraulic loading. Diurnal fluctuations in water quality within tanks such as those described by Rosenthal et al. (1981; 1982), Honer et al. (1987a) and Poxton and Allouse (1987) have been well documented. Parameters which they describe as variable, primarily total ammonia, nitrite,

pH, water temperature and DO concentration, may become critical at certain times of the day, due to a reduction in the buffering or diluting volume of water.

Shallow water depths may not be advantageous for behavioural reasons. Vertical animal movement will be impaired, which may affect feeding behaviour. Also flow rates across the reduced cross section of water within the tank may be increased above speeds greater than those preferred by the fish. Less time will be available for sinking pellets to be eaten and the proximity of the water surface in shallow tanks may be stressful because light penetration will be greater and the fish may fear being stranded.

A study of the influence of water depth on the biology of turbot, water quality in the tanks and hydrodynamics was therefore conducted with the aim of establishing if a reduction in the depth of water in the treatment tanks was beneficial. In doing so, the parameters influencing turbot growth were quantified so that the importance of beneficial and detrimental aspects could be established.

Stocking density is an important aspect which is known to influence water quality (Honer et al., 1987a, Poxton and Allouse, 1987) and hydrodynamics (Burley and Klapsis, 1985) in culture tanks. This can however be measured in two main ways: in terms of biomass per unit

volume (kg m^{-3}) which is often quoted for salmonids (Sedgwick, 1988) or biomass, or number of animals, per unit surface area of substrate (kg m^{-2} or number m^{-2}) which is often quoted for prawn ponds (Sandifer and Smith, 1975). The latter may be considered particularly suitable for turbot, however stocking density has usually been quoted in terms of volume (Purdom et al., 1972; WFA, 1975b; 1979; Brown, 1977; Hull and Edwards, 1979; Thain, 1979; Jones et al., 1980). Alteration of water depth within a particular tank, will obviously alter volume, as a factor of depth. Stocking density calculated in terms of volume (SDv) will therefore alter with depth though the biomass of fish remains unchanged. Stocking density calculated in terms of surface area of substrate (SDa) will be constant. If this study indicates that water depth does influence water quality, aspects of the biology of the fish or tank hydrodynamics, the influence may be due to differential stocking density. This study therefore aims to investigate this by altering depth in the treatment tanks whilst maintaining either SDv or SDa constant. This chapter is concerned with variation in depth at a constant SDa, whilst the following chapter repeats the experiments at a constant SDv.

Two forms of hatchery reared turbot have been described (Heap and Thorpe, 1987) and were recognised during this study: malpigmented and pigmented. Pigmented, or wild-type, fish have the normal mottled pigmentation on the dorsal surface which is found in the wild.

Malpigmented fish however have irregular areas of malpigmented skin. The abnormality is thought to be an artifact of the larval production process (Shelbourne, 1974; Klein-MacPhee, 1981), possibly resulting from high stocking density or nutritional deficiency. A recent study (Heap and Thorpe, 1987) suggested that malpigmented turbot were better suited to intensive culture conditions and consequently exhibited higher growth rates than pigmented turbot. The present study both allowed for and investigated this proposed difference.

8.2 OBJECTIVES

The aims of the investigations conducted in Water Depth Experiment 1 (WDE1) were:

1. To quantify the influence of water depth, in a defined culture tank, on the biological aspects of turbot, ie. growth rate, conversion efficiency, condition factor and behaviour, and water quality at a constant SDa.

2. To determine the inter-relationships between the biological, water quality and hydrodynamic parameters at different depths of water at a constant SDa.

3. To produce summarizing data and statements for use in the tank design model (Chapter 10).

8.3 MATERIALS AND METHODS

8.3.1 Summary of method

Experiments were set up in which turbot were grown in a range of water depths in the treatment tanks used throughout this study. All tanks contained turbot at the same stocking density, calculated as a factor of available floor area. Six of the tanks, with a range of water depths, were maintained at the same flow rate and six tanks, with a replicate range of water depths were maintained at the same residence time. A comparison of data from different treatments was made to investigate the influence of depth on various biological, water quality and hydrodynamic parameters whilst allowing for other, possibly conflicting variables, ie. stocking density, flow rate and residence time, which could not be maintained the same in all the treatments.

8.3.2 Treatments

The experiment was conducted during a 71 day period from 12/11/87 (day 0) to 21/1/87 (day 70). The recirculation system described in section 4.1.1 (Figure 4.1) was used. Six treatment tanks, described in section 4.1.2, were filled with seawater to depths of either 3, 6, 9, 12, 15 or 18 cm and flow rates were a constant 2 l min^{-1} into each tank. Six remaining tanks were kept at the replicate depths, but with a constant residence

time (ideal mean residence time, t_i) of 10.8 min. The total flow rate through both sets of six tanks was equivalent, at 12 l min^{-1}. Flow rates and residence times are summarized in Table 8.1.

Table 8.1: Treatment tank experimental regime

Tank No.	Depth (cm)	Flow (l min^{-1})	Residence time (min)
1	3	0.57	10.84
2	6	1.14	10.84
3	9	1.71	10.84
4	12	2.28	10.84
5	15	2.85	10.84
6	18	3.42	10.84
7	3	2.00	3.09
8	6	2.00	6.18
9	9	2.00	9.27
10	12	2.00	12.36
11	15	2.00	15.45
12	18	2.00	18.54

Tank volume = 2.06 l cm $depth^{-1}$

8.3.3 Fish

Post-weaned turbot within the size range 2.3 - 10.9 g were supplied by Golden Sea Produce of Hunterston, Scotland (see section 4.3). Fish were acclimated to the recirculation system for 14 days prior to the start of the experiment. During this period the stock of fish were kept in two of the treatment tanks described in section 4.1.2, filled to a depth of 16 cm. The flow rate into each tank was maintained at 4 l min^{-1}. No acclimation to the treatment conditions was carried out because it was

considered that the effect of treatment on the fish, particularly near the beginning of the experiment, may be more evident as a consequence.

A SDa of 10 fish $tank^{-1}$ was set up in each tank. Fish were assigned to each tank randomly using random number tables (Murdoch and Barnes, 1970). The tank floor area was the same in each tank, so a constant initial SDa, measured in g cm^{-2}, was assumed to occur in all tanks. Five malpigmented and five pigmented fish were assigned to each tank, so a stratified random sample method was used to assign 120 fish (10 fish $tank^{-1}$ x 12 tanks) to the treatment tanks, from a stock of 151 fish.

8.3.4 Feed

Deniel (1976) showed that a multiple feeding regime for juvenile turbot produced only active food consumption during the first feed of the day, followed by a reduced intake during subsequent feeds. Fish were therefore fed by hand to excess once daily, normally between 12.00-14.00 h. It was ensured that all fish, including sub-dominants, were given the opportunity to feed. Uneaten pellets were siphoned out of the tank usually between 1-2 h after feeding. This lag between feeding and cleaning was a compromise between maintaining a clean environment and allowing the fish to partially digest the food, so that pellets were not expelled during a stressed escape response during cleaning. The order in which the fish

were fed and tanks were cleaned was varied randomly. Feed composition, based on a diet suggested by Calcedo Juanes (pers. comm.) is shown in Table 4.3. A constant 1.5 cm long, 3 mm diameter pellet weighing 0.217 g, was produced as described in section 4.4.1. This initially proved too long for the fish to ingest, so the pellets were halved in length. The wet weight of food eaten by the fish in each tank was calculated and noted daily, as described in section 4.4.2.

8.3.5 Measurements and calculations

At the beginning of, and every 2 weeks throughout the experimental period of 10 weeks, the wet weight and total length of each fish was measured, using a method designed to reduce stress (section 4.5.1). The tank number and pigmentation type corresponding to these parameters was noted. Biomass, biomass change since previous measurement, growth rates, condition factors, and conversion efficiencies were calculated, as described in sections 4.5.1 - 4.5.5.

In an attempt to develop a method of fish identification which involved a minimum of stress, individual fish were identified at the start of the experiment using photography. Full frame macro photographs, taken with an Olympus OM10 through a Hanimex 28 mm lens onto ASA 100, 35 mm Kodak film, were taken of each fish. Upon development of the black and white

prints, distinguishing features such as pigmentation pattern, fin shape and any abberations were noted and used to identify each fish. Only features unique to the fish in each separate tank were required rather than uniqueness throughout the whole stock. This method of identification did not however prove successful because pigmentation pattern and fin shape changed during the experimental period. Photography as a means of identifying individual fish was not therefore considered appropriate in this context.

It was thought that individual fish could be identified by the rank of their weights, particularly at the extremes of the scale. This method was not however adopted because many fish had similar weights, so the technique was not considered reliable in all cases. Growth and changes in size and condition data therefore could not be attributed to individual fish.

In an attempt to simplify the method of data analysis of the biological parameters, a similar sequence of analyses were adopted throughout, with minor variations if required. The general scheme employed was:

1. Tests for normality of data.
2. Analyses to assess the influence of pigmentation on biological parameters.
3. Examination of time sequences of data.

4. Analyses to assess the influence of water depth on biological parameters in tanks with either the same residence time or the same flow rate.
5. Analyses to assess the influence of water depth on biological parameters in all tanks.

Minor variations of this method were required, due to the format of the data, eg. specific growth rate was available only as tank mean values, while individual fish weights were known.

Wherever possible, the loss of results on combination was avoided by the retention of separate data, eg. individual pigmented and malpigmented fish weights were analysed, rather than a tank mean, or two pigmentation type mean values, to assess the influence of water depth on weight distribution.

8.3.6 Water quality

To assess differential water quality levels and changes in the experimental system, four series of water quality samples were collected as described in section 4.6 and analysed using the method detailed in section 4.6 and Appendices 1 and 2.

Overall samples

Daily throughout the experimental period, water was

sampled from the common outlet from the culture tanks, at 11.30 h. Water samples were drawn from the waste gutter using a siphon tube. Care was taken not to introduce air into the tube. Both ends of the tube were held under the water to prevent artificial raising of DO levels. Temperature, DO and pH were measured within 10 min of the sample being taken. The salinity of these water samples was measured on Monday, Wednesday and Friday of each week. Salinity was maintained at approximately 35 ppt by the addition of measured quantities of dechlorinated freshwater placed into the sump tank within 1 h of the salinity measurement, to make up for evaporation losses. Additions of freshwater in excess of 16 l were added over a 6 h period to ensure mixing and reduce osmotic stress to the fish and nitrifying bacteria.

24 h samples

To indicate fluctuations in water quality in the treatment tanks, samples were taken over a 24 h period between 20.00 h. on day 24 and 20.00 h. on day 25. Samples were taken from 0.5 cm above the floor in the centre of tanks 1, 3, 6, 7, 9, and 12. Samples from the head tank outlet pipe leading to the treatment tanks were used as an indication of the background, recirculation system water quality. Temperature, pH and DO were measured hourly, on the hour, throughout the 24 h period. Unfiltered samples were taken every 4 h for later analysis of ammonia and nitrite. Previous studies (Poxton and

Allouse, 1987 and Honer et al., 1987a) have indicated that the greatest fluctuations in water quality occurred soon after feeding. Fish were fed at 12.30 h. and cleaned at 13.30 h to fit in with the sampling regime, so hourly samples taken on the hour for NH_4 and NO_2 analysis were taken between 12.00 h and 16.00 h. Table 8.2 summarizes the sampling regime.

Table 8.2: 24 h water quality sampling protocol for turbot fed at 12.30 h during WDE1 (day 24-25)

Time (h)	temp/pH DO	NH_4-N NO_2-N	Time (h)	temp/pH DO	NH_4-N NO_2-N
20.00	*	*	09.00	*	
21.00	*		10.00	*	
22.00	*		11.00	*	
23.00	*		12.00	*	*
24.00	*	*	13.00	*	*
01.00	*		14.00	*	*
02.00	*		15.00	*	*
03.00	*		16.00	*	*
04.00	*	*	17.00	*	
05.00	*		18.00	*	
06.00	*		19.00	*	
07.00	*		20.00	*	*
08.00	*	*			

* = sample taken

A sampling technique similar to that described in the within tank sample series was adopted. At each sample time, water in the treatment tanks was sampled in a random order, followed by the head tank water. Samples for later NH_4 and NO_2 analysis were sealed and stored in a freezer at less than -15^{o}C, as soon as possible.

4 h samples

It was stated previously that the greatest fluctuations in water quality and most extreme values were expected in the period between 12.00 - 16.00 h. assuming that feeding was at 12.30 h. and cleaning at 13.30 h. Critical values in water quality parameters, particularly NH_4, NO_2 and DO, were therefore expected within 3.5 - 4 h after feeding, as described by Poxton and Allouse (1987). For this reason the part of the 24 h sampling experiment between 12.00 - 16.00 h. was repeated on day 69 to further examine and verify previous results. DO, pH and temperature were measured in tanks 1, 6, 7, 12 and the head tank, on the hour during this period. Replicate samples were also taken and again stored frozen, at less than -15^{o}C, for later analysis of ammonia and nitrite.

Within tank samples

Within tank water quality variation was measured in samples collected at approximately 11.30 h. on day 4. The location of the sample points was chosen to generally indicate water quality changes as water passed through the tanks from the inlet to the outlet. A strict sampling procedure for the reduction of cross-contamination was developed. To avoid influencing samples collected later, downstream samples were taken first and upstream samples last. Plastic gloves were worn throughout the procedure

to avoid contamination, due to handling, of samples to be analysed for NH_4.

The effluent of a treatment tank was diverted from the standpipe to an acid washed 250 ml plastic sample bottle with a screw lid, and a clean 100 ml glass beaker. Care was taken to avoid obtaining water oxygenated by falling from the standpipe overflow to the gutter, so the sample was taken at the top of the overflow. The plastic sample bottle was filled, leaving sufficient space to allow for expansion of the fluid on freezing. The bottle was sealed immediately after filling.

The second sample point was located 0.5 cm above the floor in the centre of the tank. In this way water in the vicinity of the fish was sampled, but debris on the bottom was not collected. The sample was obtained using a siphon tube. To ensure that water from a previous sample remaining in the tube, held there to retain the siphon, was not collected, a small air bubble was introduced into the tube at the beginning of the removal. Fluid leaving the tube before the bubble was discarded. The sample was then run into a 100 ml glass beaker for DO, pH and temperature measurement. Cross contamination of samples to be analysed for these parameters was less critical than for samples to be analysed for NH_4 and NO_2, so a sample was always collected in the beaker before the sample bottle. Having flushed the siphon tube with the 100 ml sample, a further approximately 200 ml was siphoned into a

250 ml plastic beaker for later NH_4 and NO_2 analysis. The bottle was sealed immediately. This procedure was repeated for each of the other treatment tanks, in a random order.

Having sampled from each of the treatment tanks, a sample was taken from the inlet. All the tanks received a communal supply so it was considered reasonable not to sample from within the tank at the inlet, where some mixing with the tank contents may have taken place. A point as near as possible to the tanks, in the single supply pipe from the head tank was required. The nearest available point of access was the upstream end of the treatment tank supply pipe, in the bottom of the head tank. Using the same procedure as that described for the tank centre sample points, water was drawn by siphon into a 100 ml beaker and a 250 ml sample bottle, which was sealed immediately after use.

After collection of all samples, the sealed plastic bottles were placed in a freezer immediately and stored at less than -15 ^{o}C. Samples in the 100 ml glass beakers were then analysed for DO, pH, temperature and salinity.

8.4 RESULTS

The biological parameters of survival, weight, length, condition factor, growth, population variance, food consumed, food conversion ratio, pigmentation and

fish behaviour were either measured, calculated or qualified during this study. Due to the large number of these parameters and the possible inter-relationships between each other and with other water quality and hydrodynamic parameters, detailed statistical analysis of data was limited to the parameters which were expected to be most sensitive to treatment, or for which the most data was available. To reduce the complexity of the results, fish weight, length, condition factor and specific growth rate were investigated in some detail. The remaining parameters were analysed in less detail unless results suggested further investigation was required.

8.4.1 Survival

Throughout the experimental period, only 1 fish died: in tank 3 on day 52. It was replaced the following day with a fish of a similar size and the same type of pigmentation. The numeric method used to correct for dead fish is described in Chapter 4. Water depths between 3 and 18 cm, under the conditions stated, therefore did not stress the fish sufficiently to cause appreciable mortalities.

8.4.2 Fish weight

Normal probability plots and histograms were drawn of the individual weights of all experimental fish for each 14 day measurement period. Both types of plot indicated

that the distribution of weights could be considered normal and therefore normal statistics were applicable. A small positive skew in the distribution was evident towards the end of the experiment, indicating a greater proportion of heavier fish than would be expected in a perfectly normal distribution. This effect was not pronounced.

Two-sample t-tests were calculated, comparing the individual weights of pigmented and malpigmented fish in each treatment tank. The null hypothesis of the tests was that there was no difference in the mean weights of the two groups of fish. Table 8.3 summarises the results of the tests, indicating the probability levels achieved. Assuming at least a 5 % probability level for the rejection of the hypothesis, only tank 5 (15 cm depth) appeared to contain fish with a weight related to the type of pigmentation. In this tank, during day 56, malpigmented fish on average weighed 1.32 - 2.36 g more than pigmented fish. A significant weight difference also existed at the beginning of the experiment. No other influence of pigmentation type on the mean weight of fish in each tank was evident.

An analysis of variance was calculated to compare the individual weights of fish of each pigment type within each tank with all other such groups at each weighing period. Results of the tests indicated that weights of fish, grouped into pigmentation types within each tank,

were not different to any other such group during the same weighing period. Also no significant differences developed throughout the experimental period. Significant differences did occur between groups on different days, indicating that a significant change in weight did occur with time.

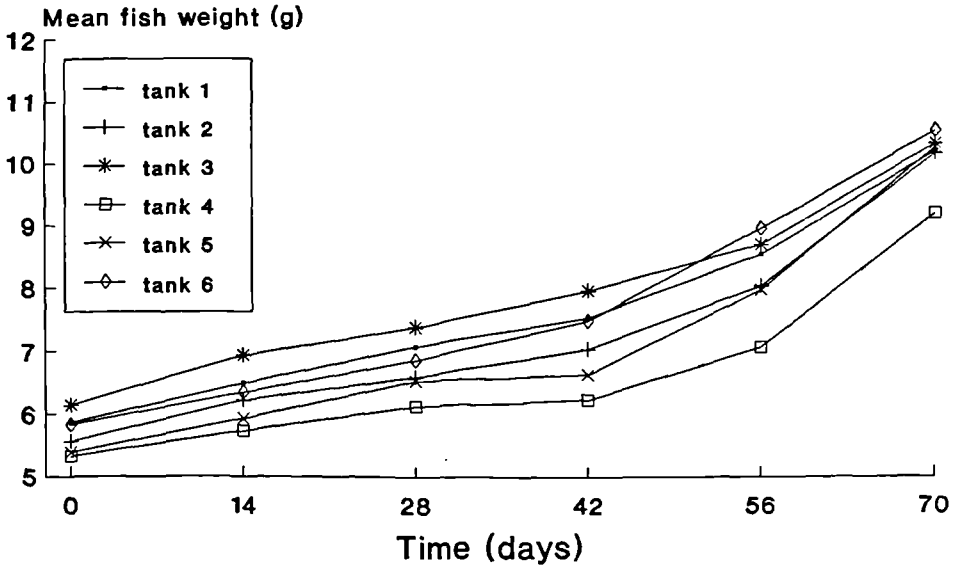
Table 8.3: Probability levels of two-sample t-tests comparing the weight of pigmented and malpigmented fish (H_0 : mean difference = 0)

Tank no.	Day 0	14	28	42	56	70
1	0.83	0.71	0.52	0.42	0.36	0.30
2	0.44	0.42	0.23	0.25	0.18	0.33
3	0.59	0.18	0.20	0.15	0.13	0.19
4	0.25	0.10	0.45	0.49	0.76	0.87
5	**0.041**	0.09	0.06	0.07	**0.045**	0.11
6	0.68	0.84	0.51	0.86	0.78	0.96
7	0.29	0.24	0.14	0.19	0.15	0.16
8	0.84	0.73	1.00	0.65	0.44	0.98
9	0.85	0.79	0.55	0.35	0.33	0.33
10	0.32	0.30	0.28	0.27	0.26	0.24
11	0.75	0.89	0.98	0.88	0.84	0.86
12	0.92	0.93	0.92	0.94	0.80	0.95

Two-sample t-test and analysis of variance results both indicated that there was no sustained difference in the mean weight of fish in any of the treatment tanks, due to pigmentation type. It was therefore considered justifiable to combine pigmented and malpigmented weights of fish from the same tank, for further analyses.

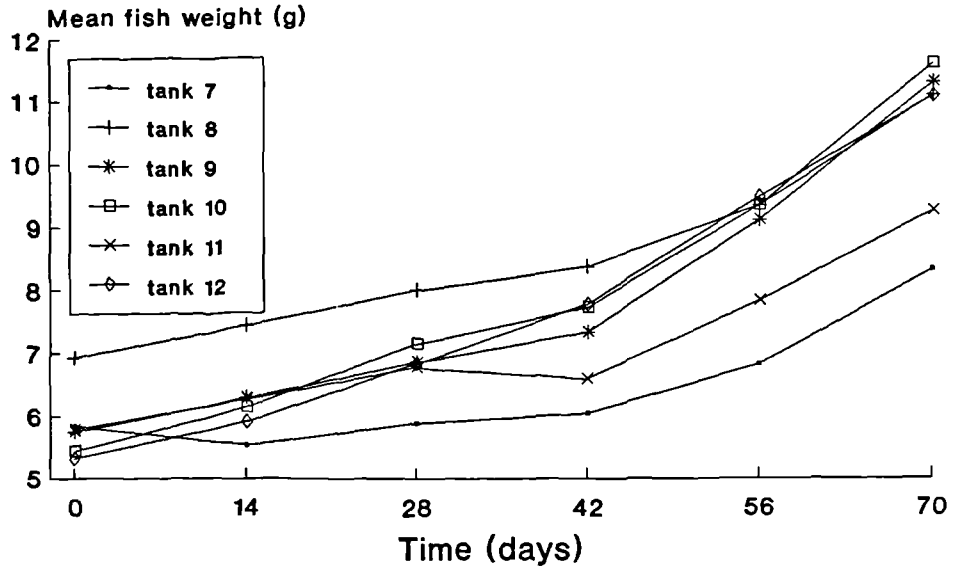
Figures 8.1 and 8.2 indicate that the mean weight of the fish in each of the tanks increased throughout the experiment. The overall mean weight of 5.72 g at day 0

Figure 8.1: Tank mean fish weight changes - same residence time tanks



Water depths: tank 1 - 3cm; tank 2 - 6cm
tank 3 - 9cm; tank 4 - 12cm
tank 5 - 15cm; tank 6 - 18cm

Figure 8.2: Tank mean fish weight changes - same flow rate tanks



Water depths: tank 7 - 3cm; tank 8 - 6cm
tank 9 - 9cm; tank 10 - 12cm
tank 11 - 15cm; tank 12 - 18cm

increased to 10.25 g at day 70. The majority of mean weight change curves were parallel, which suggested that a similar weight gain was achieved, despite differences in the treatment. However no correlation was found between final mean weight and initial mean weight ($r=0.294$, $d.f.=10$, $p>0.05$). Differences in initial mean weight in each tank were therefore unlikely to have caused differences in final mean weights. Multiple regression analysis of the influence of both initial mean weight and depth on final mean weight also indicated that no significant relationship existed ($r=0.462$, $d.f.=9$, $p>0.05$). A combination of depth and initial weight was also not therefore entirely responsible for the final mean weight.

The influence of water depth on weight of fish was investigated by analysis of variance comparing the weight distribution of all fish in a tank with the weight distribution of fish in all other tanks during the same day and within each flow type during the same day. The results indicated that at the beginning of, and throughout the experiment, the range of weights in any of the tanks was not significantly different to the range of weights in any other of the tanks, during the same weighing period. So no influence of depth on fish weight was evident, either at the beginning of the experiment or developed with time. Separation of tank results into either those with the same flow rate or the same residence time, also

produced no significant difference in weight distributions.

Results of further investigations into the influence of water depth on fish weight distribution within each tank, by correlation and regression analyses indicated that there was no significant linear association between depth and fish weight distribution at any time during the experiment. The main fish weight results are summarized in section 8.4.13.

8.4.3 Fish length

Normal probability plots and histograms were drawn of the individual lengths of all experimental fish at each day of measurement. Both types of plot indicated that the distribution of lengths could be considered normal and therefore normal statistics were applicable. Unlike the weight data, no skew in the distribution was evident.

Table 8.4 summarizes the results of two-sample t-tests comparing the individual lengths of pigmented and malpigmented fish in each treatment tank throughout the experimental period. Only tank 5 contained fish with a significant difference in length between pigmentation types. Malpigmented fish had a mean length of between 6.6 and 7.6 mm longer than pigmented fish in this tank. A decrease, with time, in the probability that the lengths of the two groups of fish were different, was observed. A

significant length difference was already present at day 0, when the populations were set up. No significant difference in length between pigmented and malpigmented fish was observed in any of the other tanks.

Table 8.4: Probability levels of two-sample t-tests comparing the lengths of pigmented and malpigmented fish (H_0: mean difference = 0)

Tank no.	Day 0	14	28	42	56	70
1	0.67	0.39	0.58	0.54	0.46	0.3
2	0.34	0.20	0.22	0.27	0.27	0.3
3	0.40	0.11	0.49	0.15	0.18	0.31
4	0.19	0.08	0.26	0.30	0.48	0.73
5	**0.025**	**0.025**	**0.021**	**0.031**	**0.045**	0.09
6	0.35	0.45	0.20	0.56	0.62	0.83
7	0.33	0.26	0.25	0.23	0.35	0.25
8	0.80	0.80	0.93	0.62	0.51	0.62
9	0.93	0.90	0.70	0.41	0.34	0.36
10	0.34	0.25	0.28	0.29	0.28	0.26
11	0.64	0.68	0.51	0.67	0.60	0.57
12	0.94	0.87	0.95	0.90	0.71	0.81

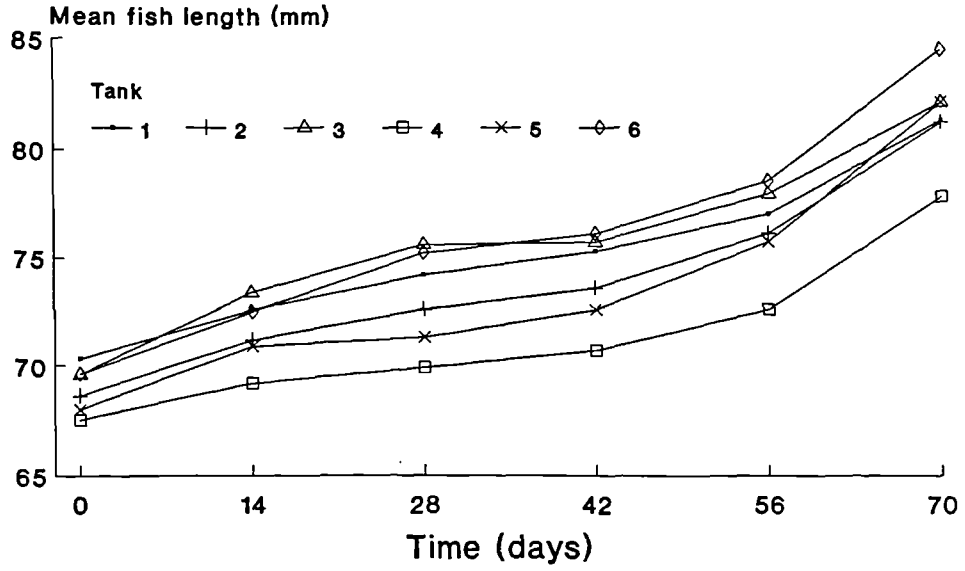
Results of analysis of variance tests comparing the distribution of fish lengths in each pigmentation group with all other pigmentation groups during the same measurement day indicated that none of the groups were significantly different to any other on the same day. Differences did not therefore increase with time. Significant differences did occur between the groups during different measurement days. This indicated a significant increase in length during the experimental period.

Only in tank 5 did significant differences in fish length, attributable to pigmentation type, occur. This difference was present at the start of the experiment as an artifact of the original assignment of fish to the tanks, and appeared to decrease as the experiment progressed. It was therefore considered reasonable, using the same method described for the weight data, to combine pigmented and malpigmented fish length data, derived from the same tank, for further analyses.

Overall mean fish length increased from 68.9 mm at day 0 to 81.5 mm at day 70 (Figures 8.3 and 8.4). Tank mean fish length increased similarly in all tanks, except tank 8 which contained marginally longer fish at the beginning of the experiment. Although lines describing mean fish length changes in the different tanks appeared parallel, final and initial mean length were not significantly correlated ($r=0.392$, $d.f.=10$, $p>0.05$), so that initial mean length was not totally responsible for final mean length. Multiple regression analysis of the influence of both initial mean length and water depth on final mean length indicated that no significant relationship existed ($r=0.462$, $d.f.=9$, $p>0.05$). The regression equation accounted for only 38 % of the variation in final mean length. A combination of depth and initial length was not therefore totally responsible for the final mean length.

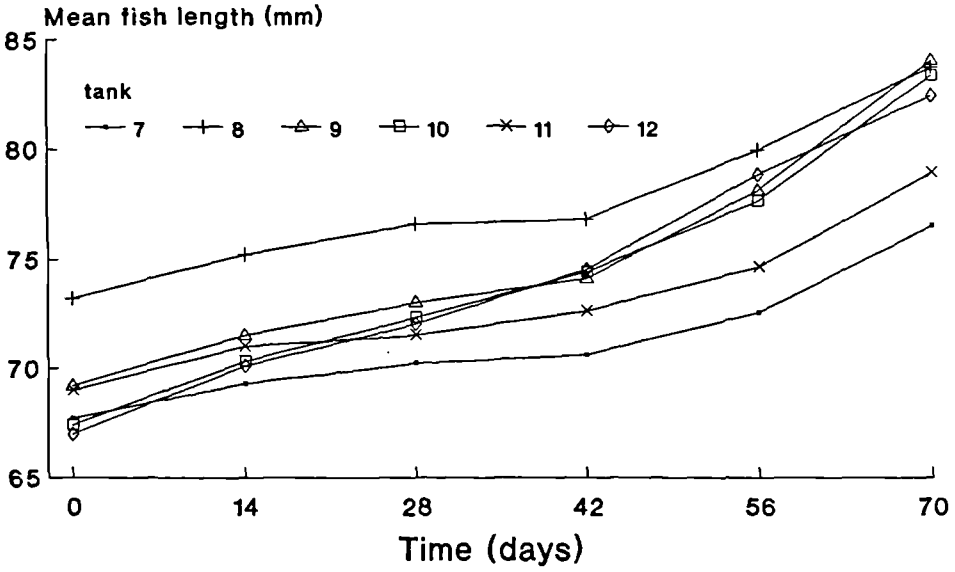
Analysis of variance tests, calculated to study the

Figure 8.3: Tank mean fish length changes - same residence time tanks



Water depths: tank 1 - 3cm; tank 2 - 6cm
tank 3 - 9cm; tank 4 - 12cm
tank 5 - 15cm; tank 6 - 18cm

Figure 8.4: Tank mean fish length changes - same flow rate tanks



Water depths: tank 7 - 3cm; tank 8 - 6cm
tank 9 - 9cm; tank 10 - 12cm
tank 11 - 15cm; tank 12 - 18cm

influence of water depth on length of fish, compared the length distribution of fish in each tank with that in all other tanks during the same day, and within each flow type group (same residence time or same flow rate tanks) of tanks during the same day. Results of these tests indicated that at the beginning and throughout the experiment, the range of fish lengths in any of the tanks was not significantly different to the range of lengths in any other of the tanks at the same measurement day. No influence of depth on fish length was therefore evident, nor did any significant differences develop with time. No significant difference in the fish length distribution, grouped into either tanks with the same flow rate or residence time, was evident.

Further investigations into the influence of depth on fish length distribution in each tank, using correlation and regression analyses indicated that depth did not significantly influence fish length distribution at any time during the experiment.

The main fish length results are summarized in section 8.4.13.

8.4.4 Condition factor

Normal probability plots and histograms of the frequency distribution of CF of the individual fish, measured each weighing period, indicated that the

distribution of CFs was generally normal throughout the experiment. The application of normal statistical methods of data analysis was therefore considered appropriate.

Two-sample t-tests were calculated, comparing the individual CFs of pigmented and malpigmented fish in each treatment tank. The null hypothesis of the tests was that there was no difference in the mean CFs of the two groups of fish. Table 8.5 summarises the results of the tests. Only in tank 3 at day 70 and tank 12 at days 14 and 28 were there significant differences in CF between the two types of fish. In tank 3, malpigmented fish had a mean CF of 0.17 greater than pigmented fish. On the other hand, in tank 12 during days 14 and 28, pigmented fish had a greater CF than malpigmented fish, by a mean of 0.26 and 0.25 respectively.

Table 8.5: Probability levels of two-sample t-tests comparing the CFs of pigmented and malpigmented fish (H_0: mean difference = 0)

Tank no.	Day 0	14	28	42	56	70
1	0.64	0.31	0.48	0.31	0.08	0.23
2	0.22	0.07	0.86	0.42	0.08	0.39
3	0.96	0.75	0.41	0.56	0.11	**0.012**
4	0.53	0.83	0.06	0.13	0.28	0.75
5	0.24	0.29	0.94	0.86	0.78	0.66
6	0.06	0.13	0.74	0.26	0.61	0.54
7	0.52	0.24	0.17	0.51	0.11	0.25
8	0.62	0.59	0.76	0.79	0.89	0.62
9	0.57	0.92	0.26	0.21	0.35	0.24
10	0.54	0.85	0.48	0.30	0.24	0.38
11	0.63	0.91	0.41	0.57	0.48	0.81
12	0.60	**0.036**	**0.007**	0.22	0.096	0.17

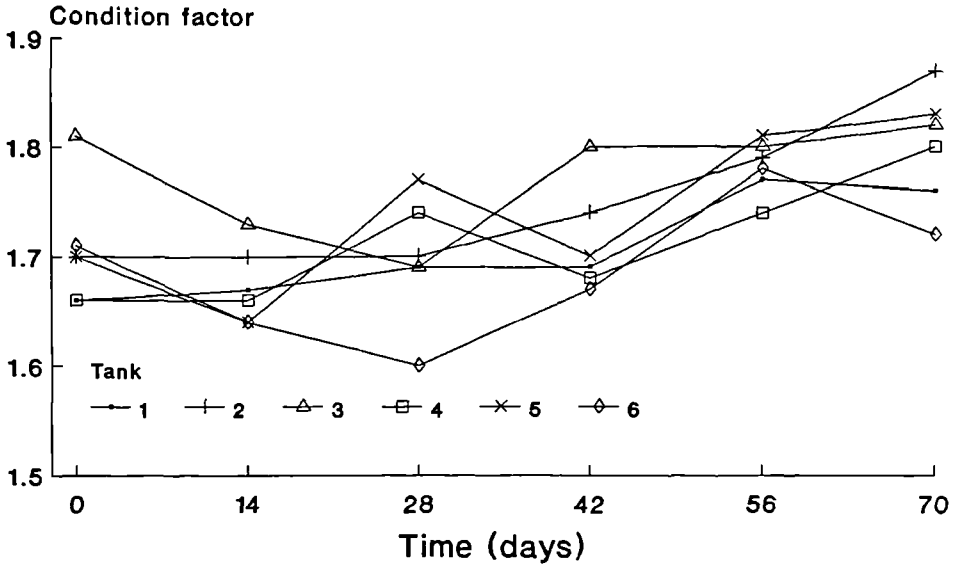
Results of ANOVA tests which were used to compare the distribution of CFs of individual pigmented and malpigmented fish within the same tank, during each weighing period, indicated that there were no significant differences in CF as a result of pigmentation type at any time during the experiment.

Results of two-sample t-tests, ANOVA, correlation and regression analyses confirm that there were no persistent or pronounced significant differences in CF due to the pigmentation type of the fish. CFs of pigmented and malpigmented fish from the same tank were therefore combined for further analyses.

Results of a paired-sample t-test comparing the difference in CF between days 0 - 70 of pigmented and malpigmented fish indicated that there was no difference between the two groups of fish ($t=1.46$, $n=12$, $p=0.17$) and that the difference between pigmentation groups did not alter significantly with depth ($r=-0.234$, $d.f.=10$, $p>0.05$).

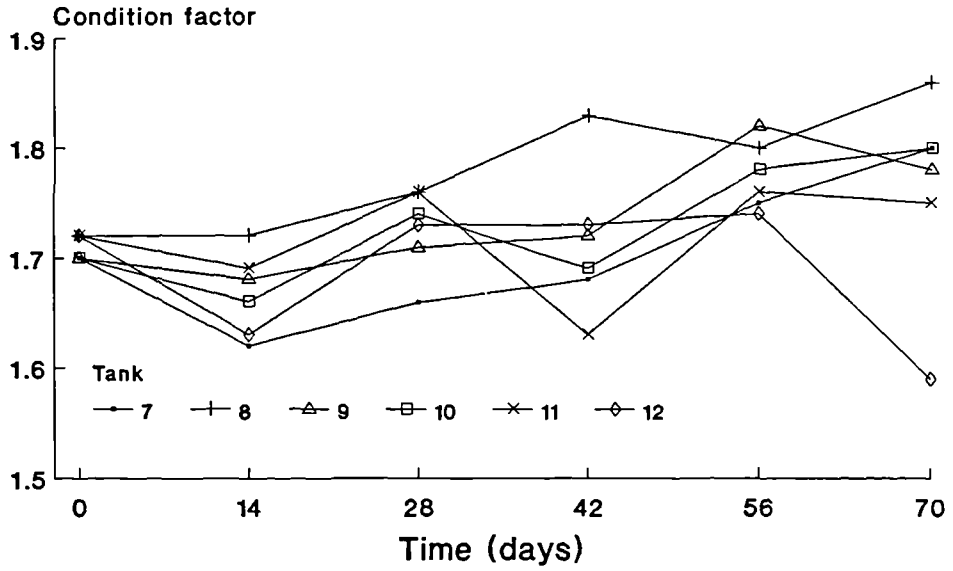
Figures 8.5 and 8.6 show the fluctuations in tank mean condition factor throughout the experimental period. CFs increased from an overall mean of 1.71 at day 0, to 1.80 at day 70. All tank mean CFs increased between days 0 and 70. A decrease in CF occurred between days 0 and 14 in 11 of the 12 tanks. No other pronounced trends were evident.

Figure 8.5: Tank mean fish CF changes - same residence time tanks



Water depths: tank 1 - 3cm; tank 2 - 6cm
tank 3 - 9cm; tank 4 - 12cm
tank 5 - 15cm; tank 6 - 18cm

Figure 8.6: Tank mean fish CF changes - same flow rate tanks



Water depths: tank 7 - 3cm; tank 8 - 6cm
tank 9 - 9cm; tank 10 - 12cm
tank 11 - 15cm; tank 12 - 18cm

Final CF was not dependant on the initial CF of the fish in each tank: the parameters were not significantly correlated. Final CFs were similar, irrespective of the initial values.

The influence of water depth on CF of fish was investigated by ANOVA tests. The distribution of CFs of fish in a tank were compared with the CF distribution of fish in all other tanks during the same day. Also, the distribution of CFs of fish in a tank were compared with the CF distribution of fish in all other tanks, with either the same residence time or the same flow rate, during the same day. Results indicated that at the beginning of the experiment, the CF distributions in any of the tanks were not significantly different to the distribution of CFs in any other of the tanks, during the same weighing period. An homologous distribution of CFs resulting from the initial assignment procedure was therefore confirmed. Also no differences in the distribution of CFs due to depth developed with time throughout the experiment. Similarly, the maintenance of the same flow rate or the same residence time also produced no significant difference in CF as a result of depth.

Results of correlation and regression analyses investigating the influence of water depth on CFs of fish within each tank indicated that at no time during the experiment did depth significantly influence CF. The

maintenance of the same flow rate or the same residence time also produced no significant difference in CF as a result of depth.

The main CF results are summarized in section 8.4.13.

8.4.5 Specific growth rate

Paired-sample t-tests were carried out to compare the G_W of pigmented and malpigmented fish in each tank. It was not possible to determine the G_Ws of individual fish, so the differences between the mean G_Ws of the two pigmentation groups within each tank, were calculated for each growth period. Results, shown in Table 8.6, indicate that, with the exception of the period 28 - 42 days, the G_Ws of pigmented and malpigmented fish in the same treatment tanks, were not significantly different. The significant difference which developed between days 28-42 did not occur either before or after this period, or during the overall period. Between 28 - 42 days malpigmented fish grew significantly faster (0.32 % day^{-1}) than pigmented fish.

Plots were drawn to examine the influence of depth on the G_W difference between fish of different pigmentation types within each tank and to ensure that variations due to depth were not masking significant differences in the two groups. No trends in Gw differences, between fish of different pigmentation types, with depth were evident.

Table 8.6: Results of paired-sample t-tests comparing the Gw of pigmented and malpigmented fish (H_0: mean difference = 0)

Day	* Mean difference	Standard deviation	df	p
0-14	-0.177	0.360	11	0.12
14-28	0.045	0.611	11	0.80
28-42	-0.323	0.418	11	**0.022**
42-56	0.023	0.330	11	0.81
56-70	0.108	0.439	11	0.41
0-70	-0.066	0.238	11	0.36

* pigmented G_W - malpigmented G_W (% day^{-1})

Results of correlation and regression analyses examining possible relationships between depth and G_W of the fish, divided into their pigmentation groups, are summarized in Table 8.7. A significant correlation between G_W and depth occurred in two of the pigmentation type groups: pigmented fish during the period 42 - 56 days and pigmented fish during the period 0 - 70 days. An increase in G_W with increased depth, within the range 3-18 cm, was indicated. The G_W of the other pigmentation group, during the same periods, in both cases was not significantly correlated with depth. A statistical comparison between the slope values of two fitted regression lines describing the association between depth and G_W of pigmented and malpigmented type fish, during the same period, was not therefore possible. So differential growth between fish of the two pigmentation types was not shown to occur.

Only during the period 28 - 42 days was there a significant difference in the G_W of fish of the two pigmentation groups. This statistically significant difference did not extend to other growth periods, so G_W data from the two pigmentation groups within each tank were therefore combined in further analyses. To avoid a loss of data on combination of the results from the two pigmentation groups, the data were kept separate, rather than the calculation of a single tank mean value.

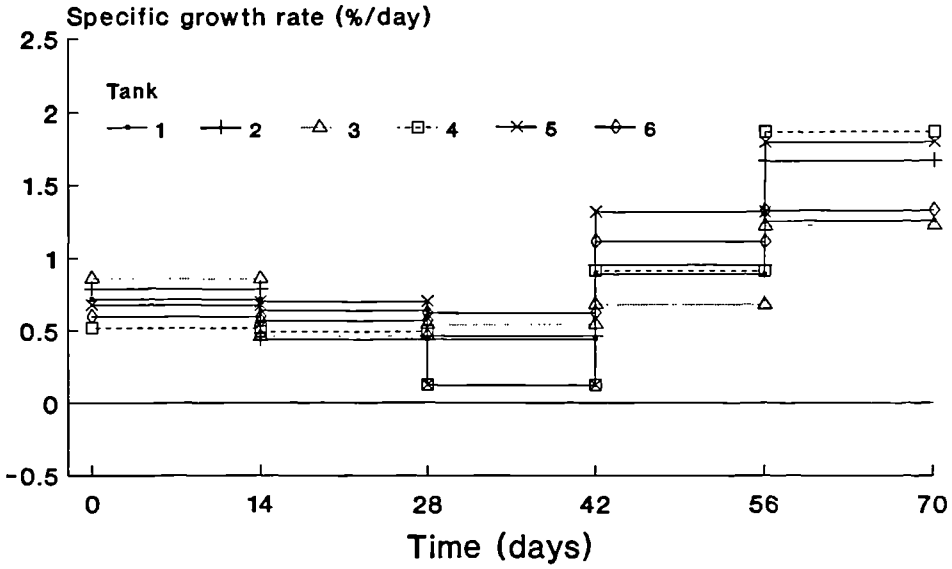
Table 8.7: Summary of results of correlation and regression analyses investigating the influence of depth on the Gw of pigmented and malpigmented fish (Gw = a + b depth)

Days	Pigm. type	r	b value	p
0-14	p	0.375	0.014	0.230
0-14	m	0.062	0.003	0.847
14-28	p	0.513	0.031	0.088
14-28	m	0.102	0.008	0.752
28-42	p	0.290	0.015	0.360
28-42	m	0.031	0.002	0.923
42-56	p	**0.703**	0.047	**0.011**
42-56	m	0.311	0.017	0.326
56-70	p	-0.088	-0.007	0.787
56-70	m	0.114	0.006	0.723
0-70	p	**0.685**	0.020	**0.014**
0-70	m	0.183	0.007	0.569

p = pigmented fish m = malpigmented fish
df = 10

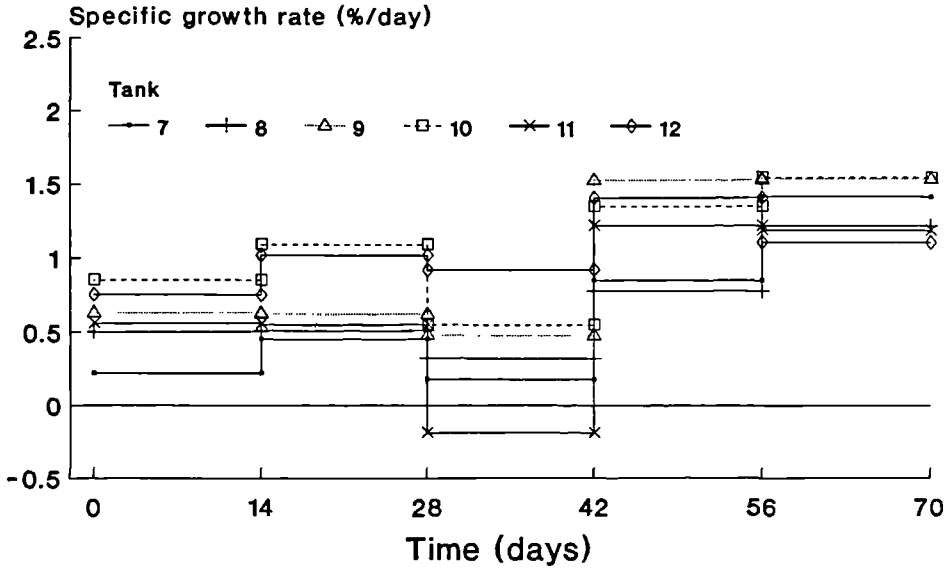
Figures 8.7 and 8.8 show the fluctuations in Gw during the experiment. Between-tank variations in G_W during each weighing day were small compared with changes between different periods. A similar pattern of

Figure 8.7: Tank mean fish Gw changes - same residence time tanks



Water depths: tank 1 - 3cm; tank 2 - 6cm
tank 3 - 9cm; tank 4 - 12cm
tank 5 - 15cm; tank 6 - 18cm

Figure 8.8: Tank mean fish Gw changes - same flow rate tanks



Water depths: tank 7 - 3cm; tank 8 - 6cm
tank 9 - 9cm; tank 10 - 12cm
tank 11 - 15cm; tank 12 - 18cm

fluctuations in each of the tanks was apparent. Growth between days 0 - 42 was fairly consistent, at approximately 0.5 - 1.0 % day^{-1}. This was followed by a period of more rapid growth between days 42 - 70. Overall mean G_w during the experiment was 0.83 % day^{-1}.

The influence of depth on G_w was investigated, whilst allowing for differences due to the maintenance of either the same flow rate or residence time in each group of six tanks. G_w was plotted against depth, during each growth period, separately for both sets of six flow type tanks. Table 8.8, summarizing the results of correlation and regression analyses of these plots, indicates that two flow type groups of fish exhibited a significant correlation between G_w and depth. G_w increased with increased depth in both cases, ie. in tanks with the same flow rate between days 0 - 14 and days 42 - 56 (Figures 8.9 and 8.10). Examination of the slope values (b) of the regression, quantifying the association between depth and G_w, of these two groups indicated that they were significantly different from zero, showing that depth and G_w were associated, and were not significantly different from each other. r and b values for the same group of fish during periods between the two significant results were not significant. The regression equation for the period 42 - 56 days indicated that a 15 cm increase in depth, from 3 to 18 cm in the treatment tanks, produced a mean increase in G_w of 0.57 % day^{-1}, from 0.893 to 1.463 % day^{-1}.

Figure 8.9: Influence of water depth on the Gw of fish held at the same flow rate during the period 0-14 days

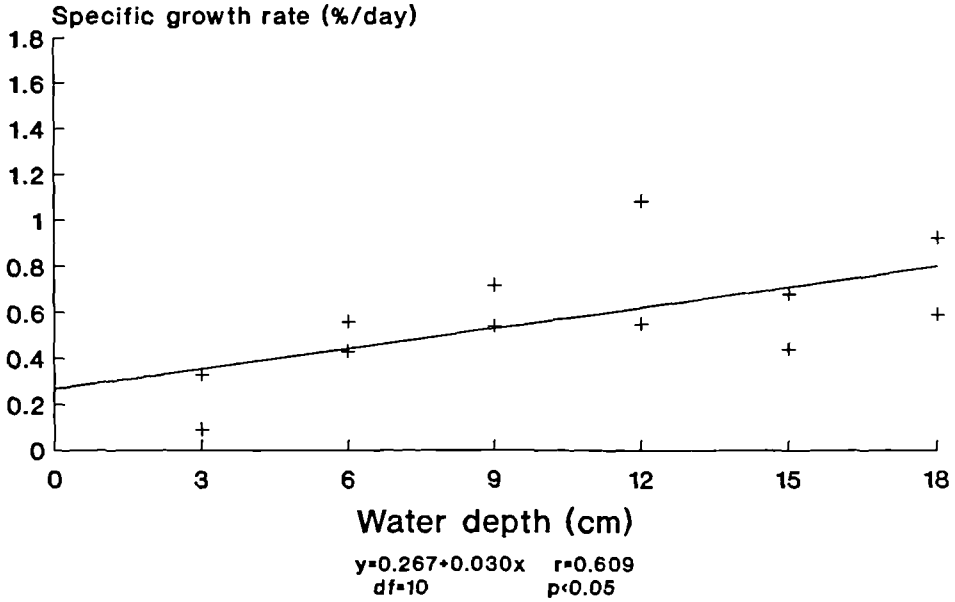


Figure 8.10: Influence of water depth on the Gw of fish held at the same flow rate during the period 42-56 days

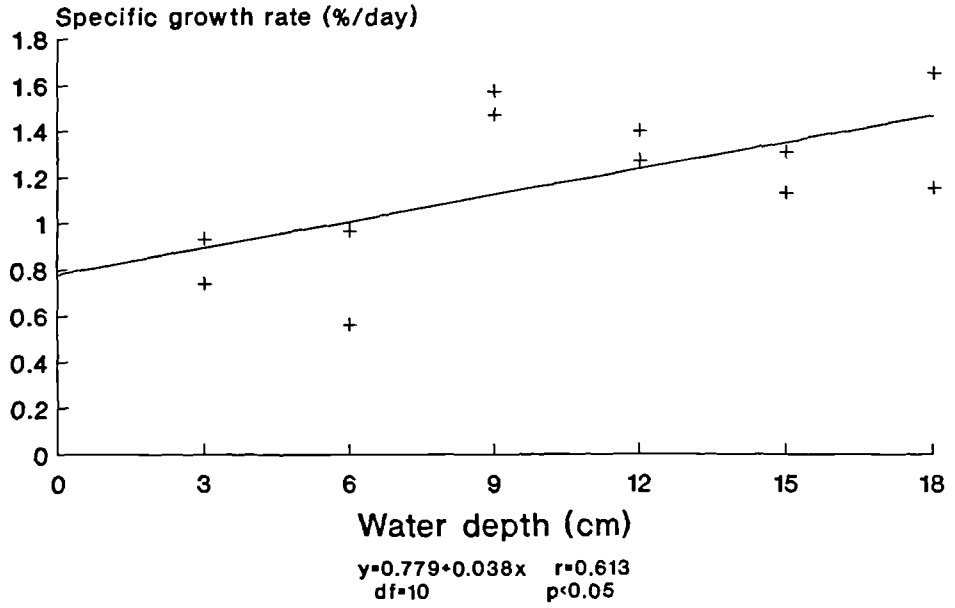


Table 8.8: Summary of results of correlation and regression analyses investigating the influence of depth on the Gw of fish held in tanks with either the same residence time or the same flow rate (Gw = a + b depth)

Days	flow type	r	b value	p
0-14	r	-0.235	-0.012	0.462
0-14	f	**0.609**	0.030	**0.036**
14-28	r	0.073	0.005	0.822
14-28	f	0.534	0.033	0.074
28-42	r	-0.063	-0.004	0.845
28-42	f	0.248	0.021	0.438
42-56	r	0.490	0.025	0.105
42-56	f	**0.613**	0.038	**0.034**
55-70	r	0.218	0.014	0.496
56-70	f	-0.276	-0.015	0.385
0-70	r	0.224	0.006	0.484
0-70	f	0.504	0.021	0.095

Flow type: r = same residence time
f = same flow rate
df = 10

The influence of depth on specific growth rate, irrespective of pigmentation or flow type group, was investigated. The mean G_ws of the four pigmentation and flow type groups of five fish each, grown at each depth, were plotted against depth. Correlation and regression analyses were also calculated to quantify any associations. Results of the tests are shown in Table 8.9. Depth significantly influenced G_w during the period 42 - 56 days as described by the equation $y=0.744+0.032x$ ($r=0.524$, $d.f.=22$, $p<0.01$). A 15 cm increase in water depth from 3 - 18 cm increased G_w by a mean of 0.48 % day^{-1} from 0.84 to 1.32 % day^{-1}. This was the only period

during which a significant association between G_W and depth was apparent.

Table 8.9: Summary of results of correlation and regression analyses investigating the influence of depth on the Gw of all fish (Gw = a + b depth)

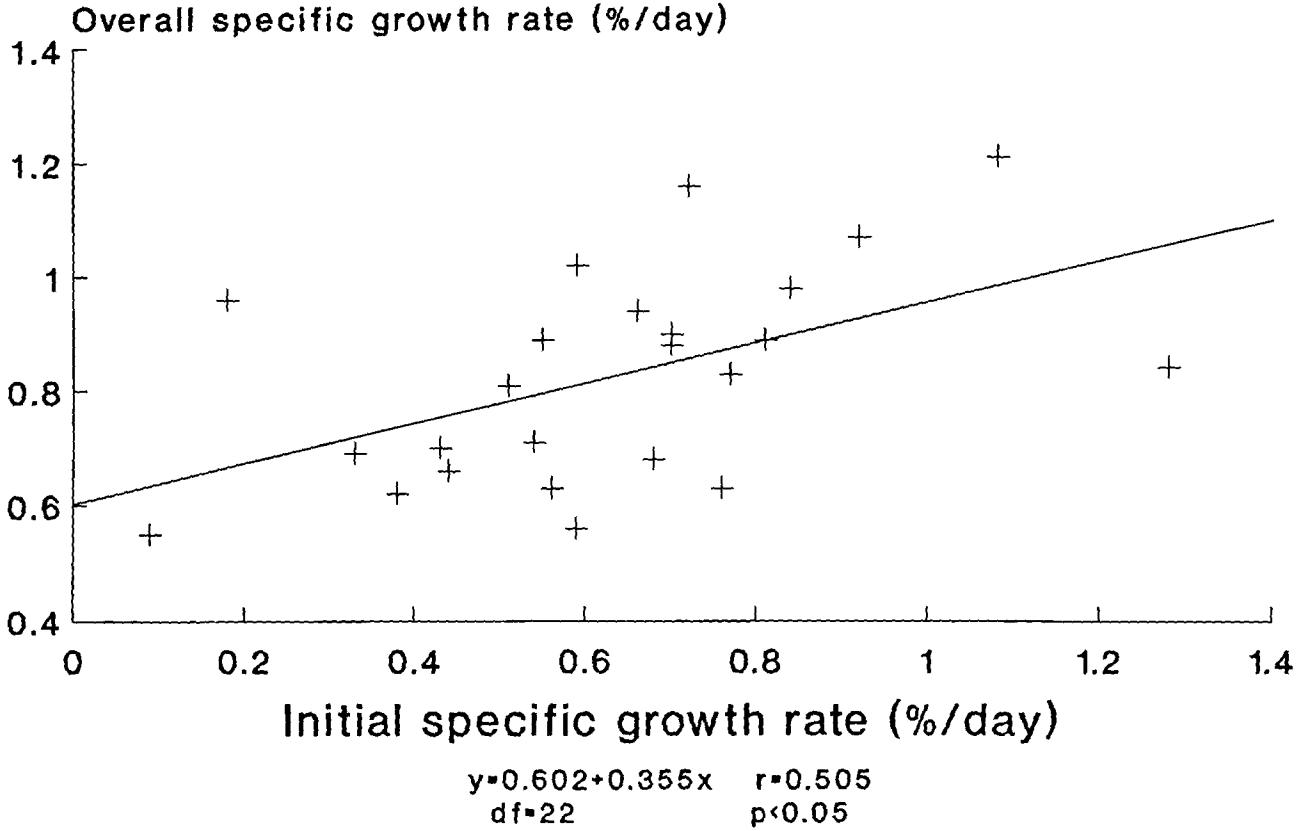
Days	r	b value	p
0-14	0.176	0.009	0.411
14-28	0.281	0.019	0.184
28-42	0.118	0.009	0.582
42-56	**0.524**	0.032	**0.009**
56-70	-0.008	-0.001	0.971
0-70	0.386	0.014	0.062

df = 22

Initial and final G_W values of fish from each tank were not significantly correlated, indicating that the final growth rate achieved was not a consequence of an initial stocking bias. However initial G_W (days 0 - 14) and overall G_W (days 0 - 70) (Figure 8.11) were significantly correlated, as described by the equation $y=0.602+0.355x$ ($r=0.505$, d.f.=22, $p<0.05$). The validity of this latter test is however suspect as the x and y parameters were not independent: initial G_W formed part of the period which comprised the overall G_W. Similarly, overall G_W (days 0 - 70) and the G_Ws during the days 14-28, 28 - 42 and 42 - 56, were significantly correlated ($r=0.735$, 0.559 and 0.671 at d.f.=22, respectively).

Finally the associations between G_W values during

Figure 8.11: Influence of initial (days 0-14) on overall (days 0-70) Gw of fish in all tanks



consecutive periods were examined. G_W during the days 14 - 28 and 42 - 56 were significantly correlated ($r=0.495$, $d.f.=22$, $p<0.05$).

The main Gw results are summarized in section 8.4.13.

8.4.6 Coefficient of variance

Coefficient of variance (Vu) can be used to measure the variation of weights within a specified group of fish as described in section 4.5.4. This measure was considered to be most appropriately applied to the tank mean level of data analysis.

Table 8.10 indicates that Vu of fish populations in 9 of the 12 tanks increased significantly during the experiment. Vu appeared to increase with time in tank 2. In tank 5 Vu reached a maximum value at day 42. Vu appeared to decrease non-linearly with time in tank 8. Fish weights were also shown to increase with time.

Table 8.10: Summary of the significant increases in Vu of fish populations with time during the experiment

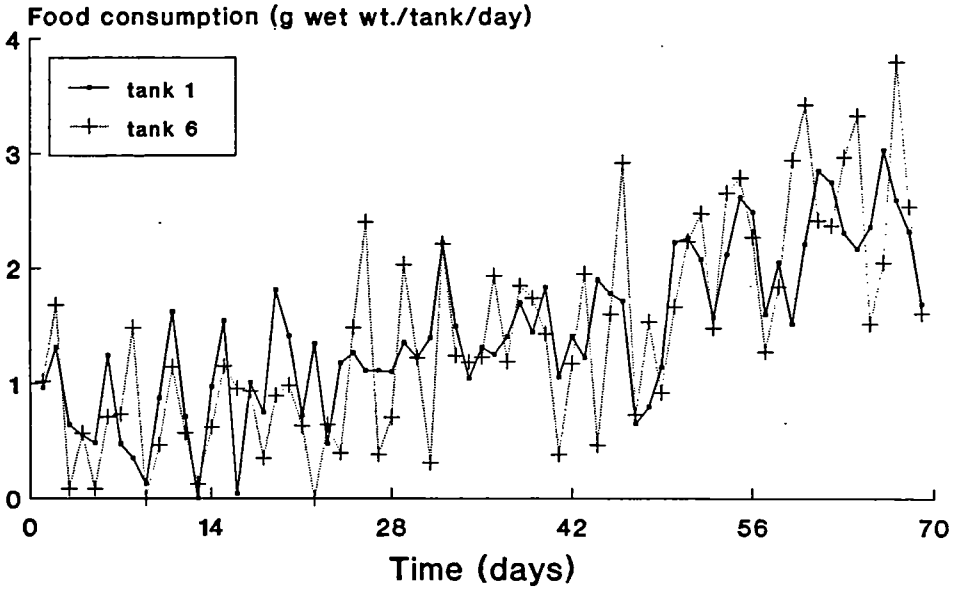
Tank no.	Water depth (cm)	flow type	r	p
1	3	r	0.990	<0.001
3	9	r	0.974	<0.01
4	12	r	0.866	<0.05
6	18	r	0.860	<0.05
7	3	f	0.975	<0.001
9	9	f	0.969	<0.01
10	12	f	0.950	<0.01
11	15	f	0.950	<0.01
12	18	f	0.987	<0.001

r = same residence time tanks
f = same flow rate tanks

8.4.7 Food consumed

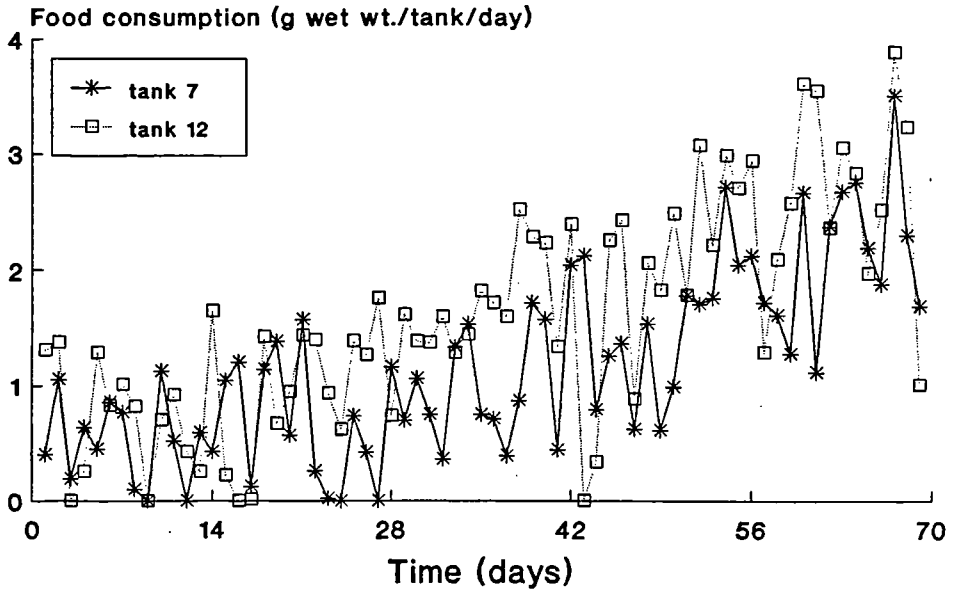
Food consumption, though variable, increased throughout the experiment, as indicated by representative examples shown in Figures 8.12 and 8.13, coincident with increased fish weight. It was not possible to calculate individual fish food consumption and therefore individual fish food conversion ratios during this experiment (section 4.5.5). No prolonged periods of increased or decreased food intake occurring simultaneously in several tanks, or associations between treatment and food intake, were apparent. Tank mean food consumptions during a growth period ranged between 0.14 - 3.38 % of body weight day^{-1} (wet/live). Mean consumption by all fish at day 14 was 0.80 g wet food $tank^{-1}$, rising to a mean of 2.36 g wet food $tank^{-1}$ at day 56, equivalent to approximate means of

Figure 8.12: Daily food consumption fluctuations - fish in tanks 1 and 6



tank 1 - 3cm depth tank 6 - 18cm depth
10 fish/tank

Figure 8.13: Daily food consumption fluctuations - fish in tanks 7 and 12



tank 7 - 3cm depth tank 12 - 18cm depth
10 fish/tank

1.27 and 2.82 % of body weight day^{-1} (wet weight/live weight) respectively.

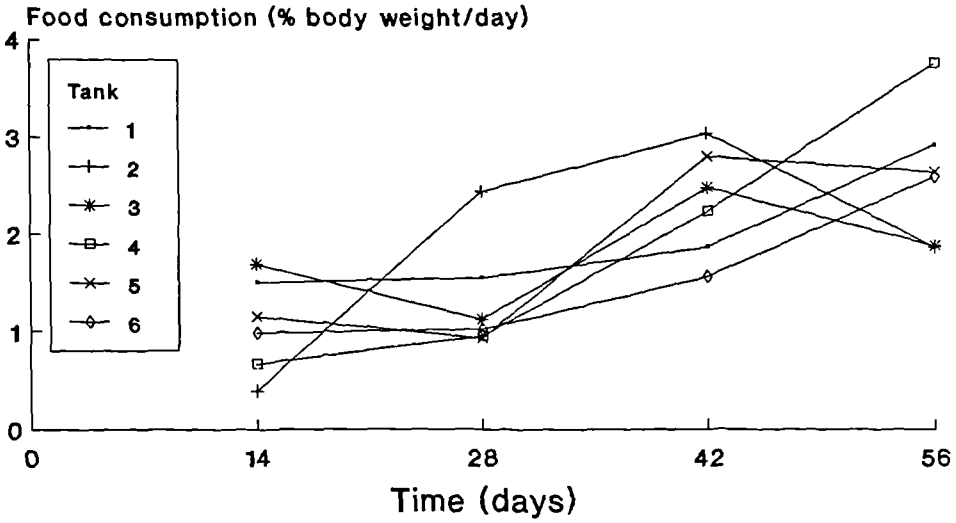
Daily food consumption fluctuations, expressed as a percentage of body weight (wet weight/live weight), are shown in Figures 8.14 and 8.15. No clear associations between the percentage of body weight consumed and either treatment tank or time, were detected.

8.4.8 Food conversion ratio

FCRs, in terms of both the wet and dry weight of food consumed, were calculated as described in section 4.5.5. Statistical analyses were carried out on dry feed weight FCRs. Wet feed weight FCRs for growing fish ranged between 1.15 - 11.58, corresponding to a dry feed weight FCR range for growing fish of 0.69 - 6.93.

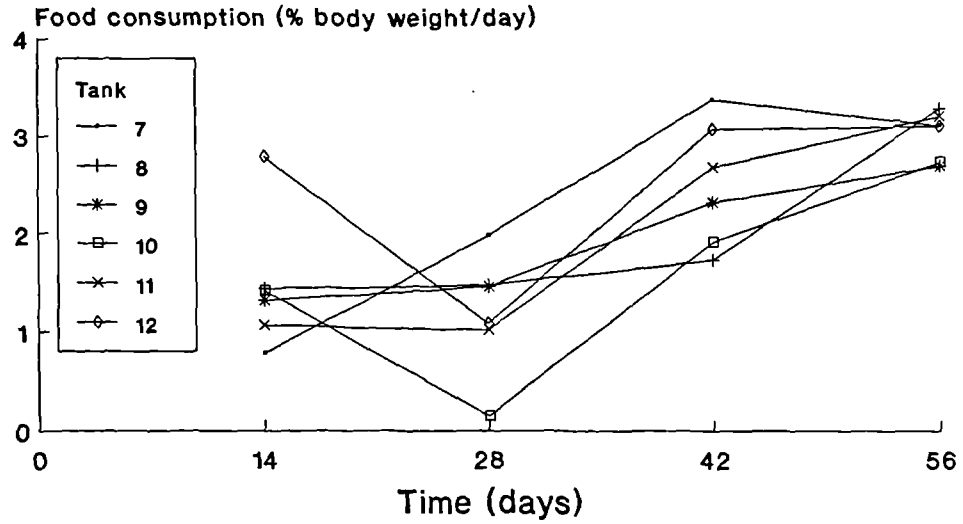
The results of correlation and regression analyses calculated to investigate the influence of water depth on FCR of fish in tanks with the same residence times, the same flow rates, or all tanks studied, are summarized in Table 8.11. The results indicate that there was a significant linear decrease in FCR with increased water depth in all tanks during the periods 14 - 28 and 42 - 56 days (Figures 8.16 and 8.17 respectively). The weight of food required to increase fish weight therefore decreased, ie. improved with increased water depth.

Figure 8.14: Daily food consumption fluctuations expressed as a percentage of body weight (wet weight/live weight)



Depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 5 - 15cm, tank 6 - 18cm

Figure 8.15: Daily food consumption fluctuations expressed as a percentage of body weight (wet weight/live weight)



Depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 11 - 15cm, tank 12 - 18cm

Figure 8.16: Decrease in food conversion ratio with increased water depth during the period 14-27 days (all tanks)

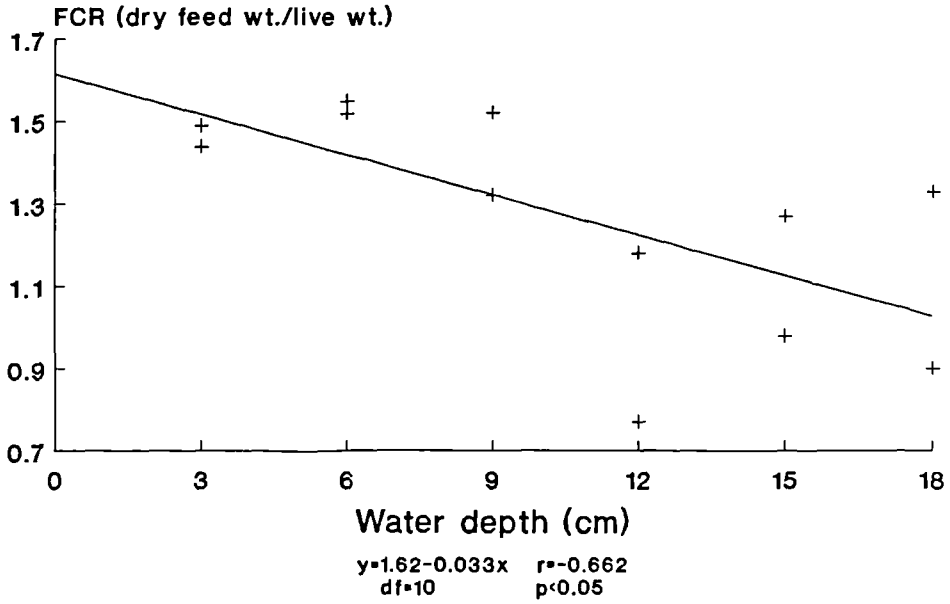
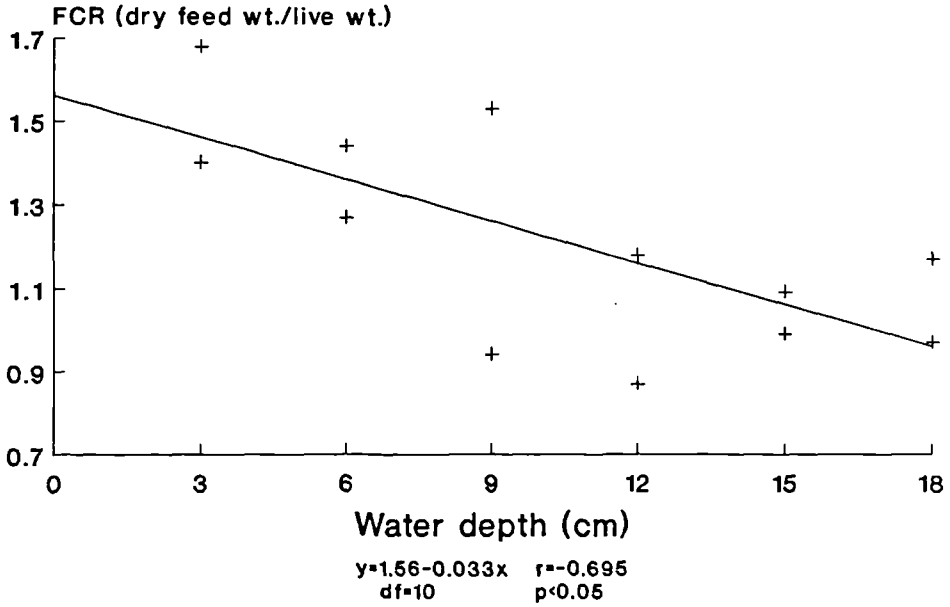


Figure 8.17: Decrease in food conversion ratio with increased water depth during the period 42-55 days (all tanks)



The main FCR results are summarized in section 8.4.13.

Table 8.11: Influence of water depth on tank mean fish food conversion ratio

Growth period (days)	tank group	d.f.	r	regression significance p
0 - 13	r	4	0.401	0.431
0 - 13	f	4	-0.763	0.078
0 - 13	all	10	-0.417	0.178
14 - 27	r	4	-0.627	0.183
14 - 27	f	4	-0.734	0.097
14 - 27	all	10	-0.662	**0.019**
28 - 41	r	4	0.337	0.513
28 - 41	f	4	-0.696	0.124
28 - 41	all	10	-0.236	0.461
42 - 55	r	4	-0.664	0.151
42 - 55	f	4	-0.804	0.054
42 - 55	all	10	-0.695	**0.012**
56 - 69	r	4	-0.167	0.751
56 - 69	f	4	0.314	0.544
56 - 69	all	10	0.068	0.833

r = same residence time tanks
f = same flow rate tanks

8.4.9 Daily water quality fluctuations

Temperature

During the experimental period, water temperature varied between 15.2 - 17.8 ^{o}C with a mean of 16.7 ^{o}C, as shown in Figure 8.18. A weekly periodicity in temperature fluctuation was apparent. 5 of the 7 marked falls in temperature occurred on a weekend, particularly Sunday.

No overall marked change in temperature throughout the experimental period was apparent.

pH

pH decreased slowly during the 70 day experiment, from 8.16 to 7.86 (Figure 8.19). No artificial buffering was required.

Salinity

Salinity was maintained approximately constant throughout the experiment by the addition of calculated quantities of dechlorinated freshwater (Figure 8.20). With the exception of one value of 33.7 ppt, salinity fluctuated within the range 34.6 - 35.8 ppt.

Dissolved oxygen

DO concentrations varied between 7.1 and 8.6 mg l^{-1}, with a mean of 7.9 mg l^{-1} equivalent to variations between 91.4 and 109.9 % saturation with a mean of 100.3 %, as shown in Figure 8.21. A marked periodicity in DO saturation, similar to that of temperature, was observed. Six of the eight main peaks in DO occurred at a weekend. A pronounced decrease in DO saturation occurred between days 18 and 19, coincident with the failure of the recirculation system water pump for approximately 4 h. Values returned to normal by day 20.

Figure 8.18: Recycle system daily water temperature fluctuations

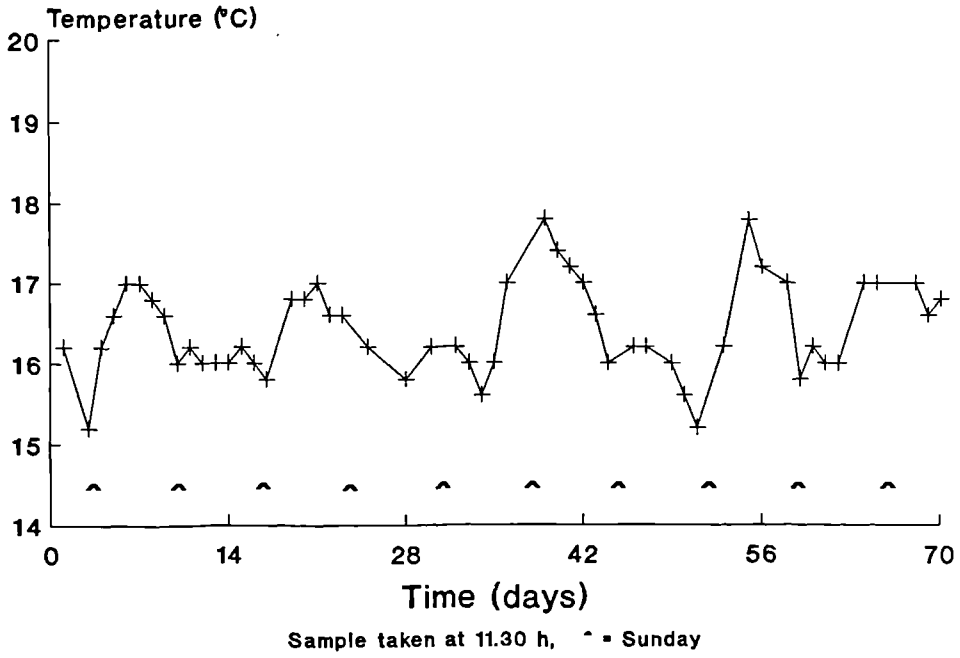


Figure 8.19: Recycle system daily water pH fluctuations

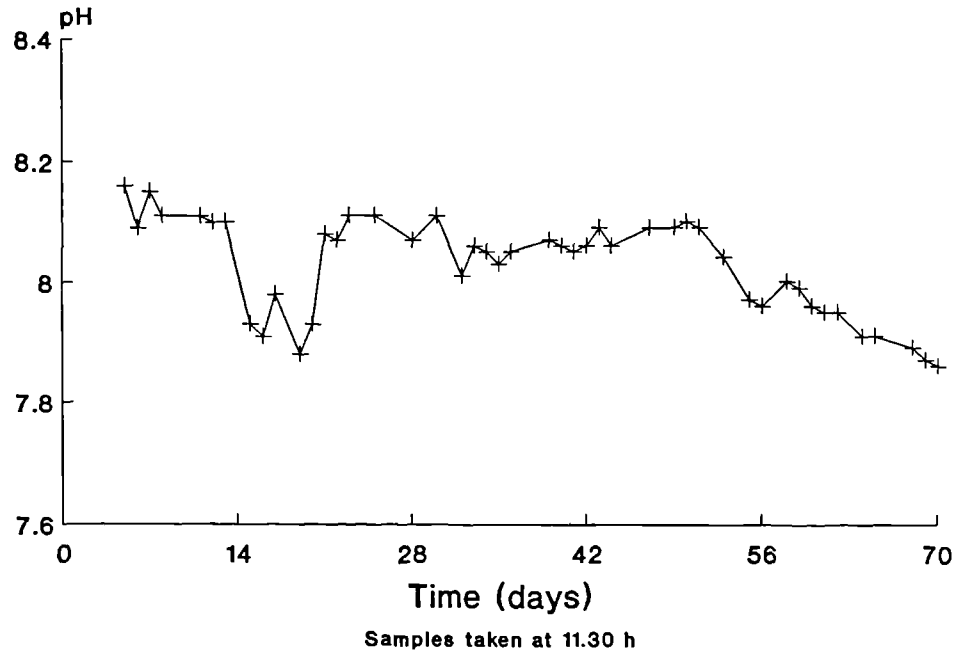


Figure 8.20: Recycle system daily salinity fluctuations

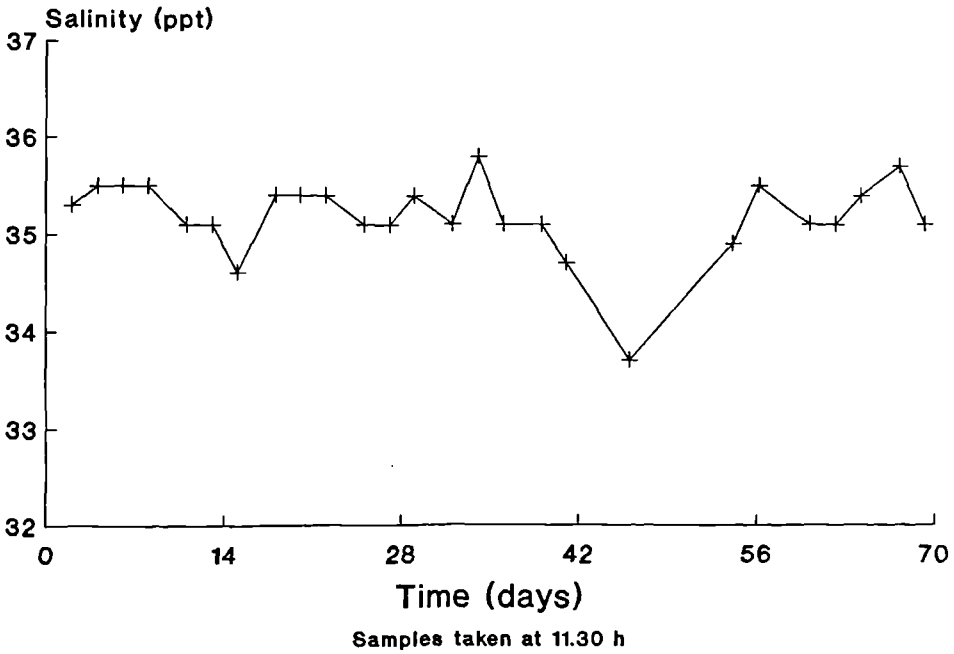
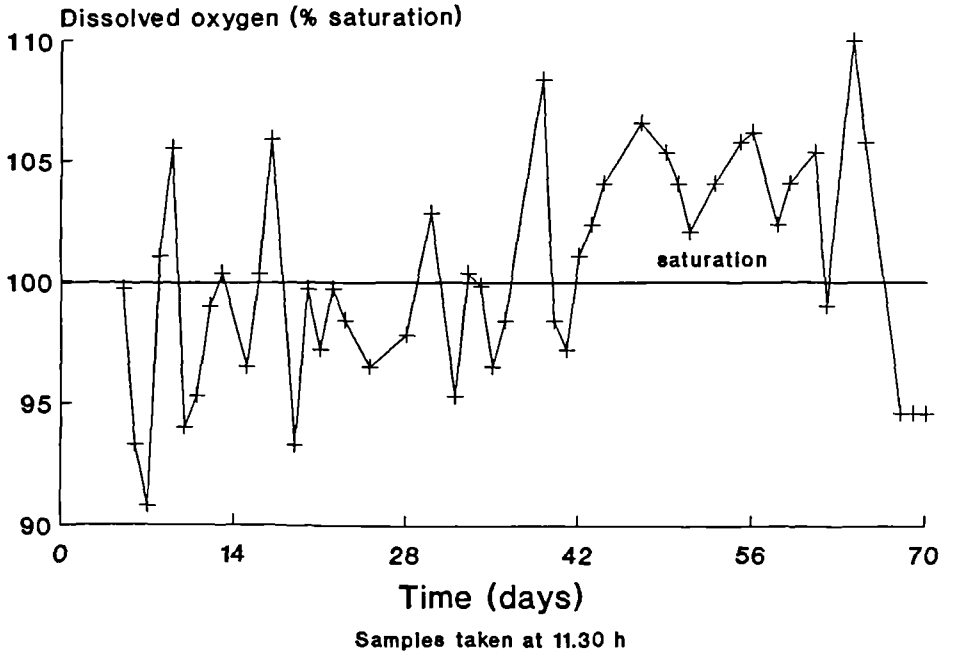


Figure 8.21: Recycle system daily dissolved oxygen fluctuations



8.4.10 24 h water quality fluctuations

Water quality in tanks 1,3,6,7,9 and 12 were investigated during days 24 and 25, as described in the methods section 8.3.

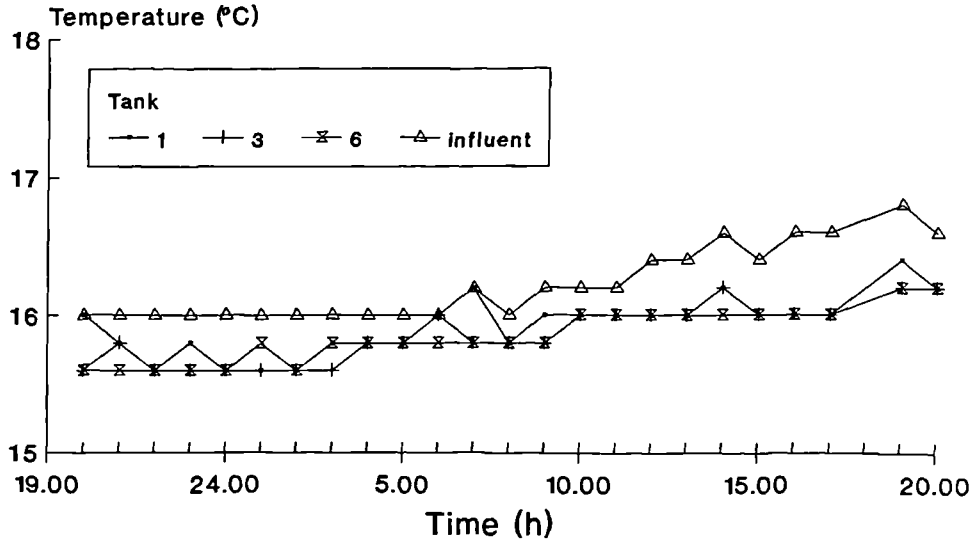
Temperature

Temperature rarely fluctuated more than 0.4 °C between each hourly sample and increased through the sample period from a mean of 15.7 °C to a mean of 16.2 °C. Figures 8.22 and 8.23 show that temperature fluctuations were similar in all tanks. No clear influence of water depth was apparent. Between approximately 20.00-07.00 h, and between 16.00 - 20.00 h, temperature remained almost constant. The rise in temperature occurred between approximately 07.00 - 16.00 h, when air temperatures were higher. The temperature of the treatment tank influent water from the head tank, shown for comparison in both figures, differs little from the temperature within the treatment tanks.

pH

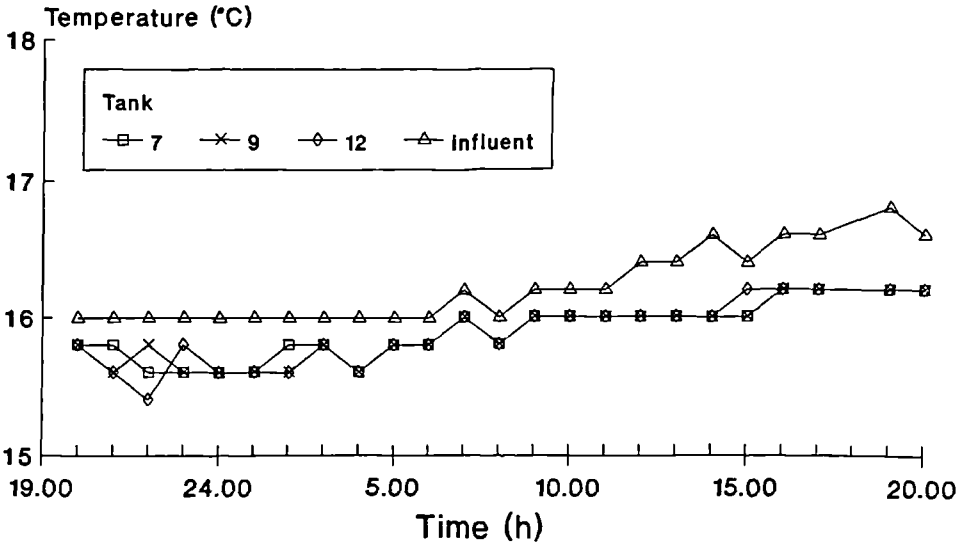
pH gradually decreased throughout the day, from approximately pH 8.12 to 8.08 (Figures 8.24 and 8.25). The difference in pH between tanks at any time was less than 0.04. A pronounced decline in pH in all treatment tanks, directly after feeding, was evident. A decrease in

Figure 8.22: Water temperature fluctuations during a 24 h period - same residence time tanks



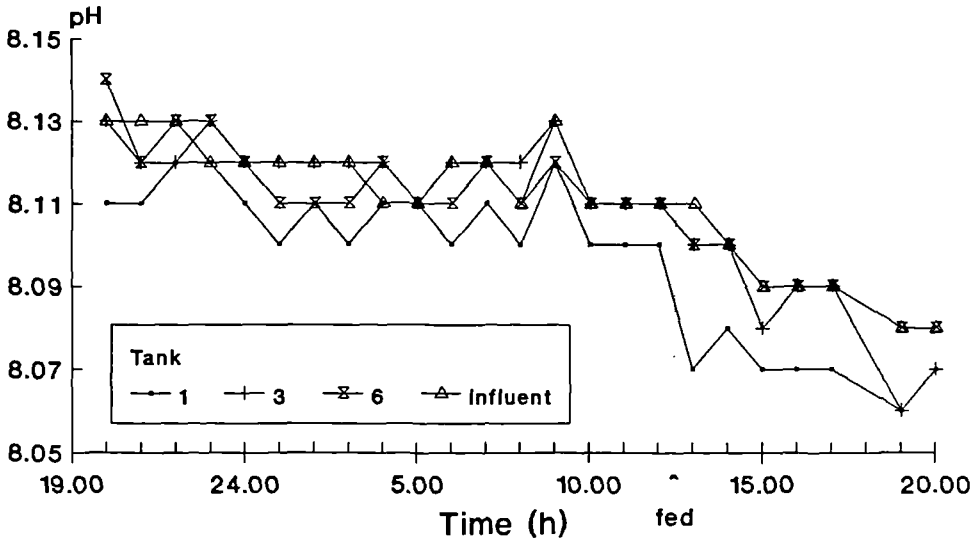
Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 12cm. Influent = head tank

Figure 8.23: Water temperature fluctuations during a 24 h period - same flow rate tanks



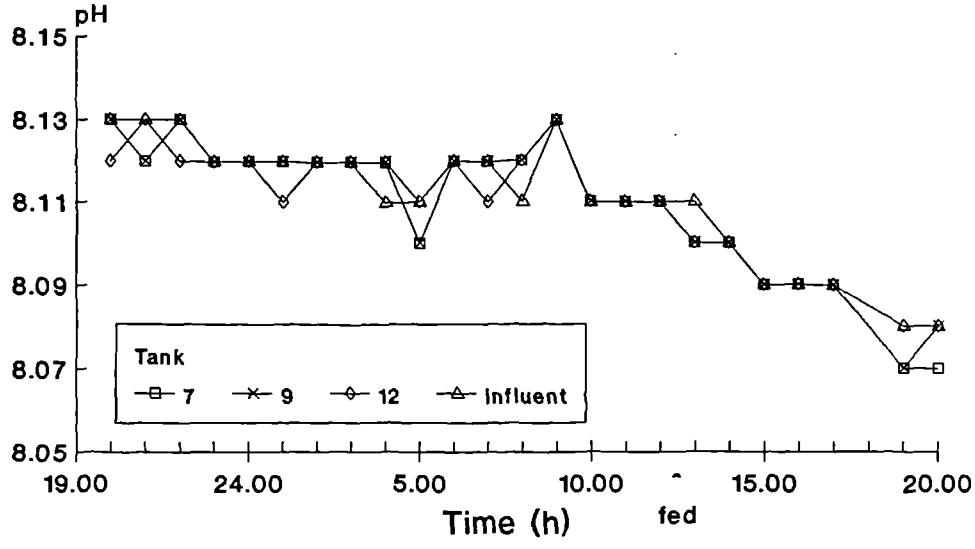
Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 12cm. Influent = head tank

Figure 8.24: Water pH fluctuations during a 24 h period - same residence time tanks



Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 18cm. Influent = head tank

Figure 8.25: Water pH fluctuations during a 24 h period - same flow rate tanks



Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 18cm. Influent = head tank

influent water pH was not detected until the following water sample period 1 h later. pH continued to fall, more slowly than the initial decline, until the end of the experiment. A small decrease in pH occurred at the beginning of the sample period until approximately 01.00 h. Between 01.00 h and the time of feeding, at 12.30 h, pH remained fairly constant. A pattern of decreasing rather than cyclical pH change was therefore demonstrated.

Figure 8.24 also indicates that tank 1 (3 cm depth) contained water with the persistently lowest pH of the three tanks of the same residence time. The most pronounced fall in pH after feeding occurred in this tank. Tank 1 contained the least volume of water and therefore had the lowest flow rate of the three tanks, to ensure a constant residence time. Figure 8.25 shows that tank 7 contained water with an equal or lower pH than the three tanks with the same flow rate during 19 of the 24 samples. The most pronounced fall in pH after feeding, in the tanks with the same flow rate, evident at 15.00 h, occurred in tank 7. Variation in pH between the three tanks and influent was however small. Tank 7 contained the least water volume of the three tanks of the same flow rate and so had the shortest residence time, ie. theoretically the greatest flushing, of the three tanks. The common factors between the 2 tanks with the lowest and fastest changing pH was therefore shallow water depth and low volume, not flow rate or residence time. In tanks 1 and 7 water flows

were 0.57 and 2.0 l min^{-1}, whilst residence times were 10.84 and 3.09 min respectively.

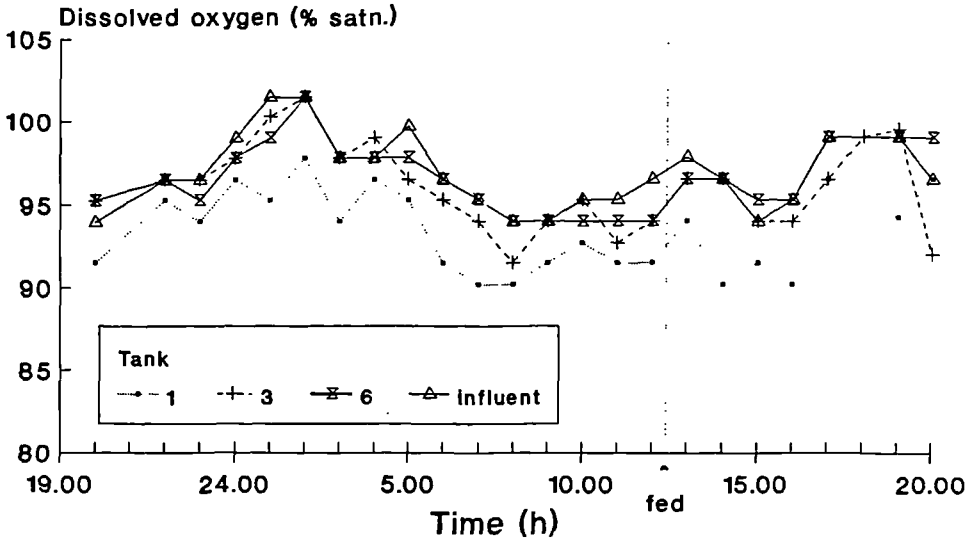
Salinity

A constant salinity of 35.1 ppt was maintained, with no detectable fluctuations throughout the sample period.

Dissolved oxygen

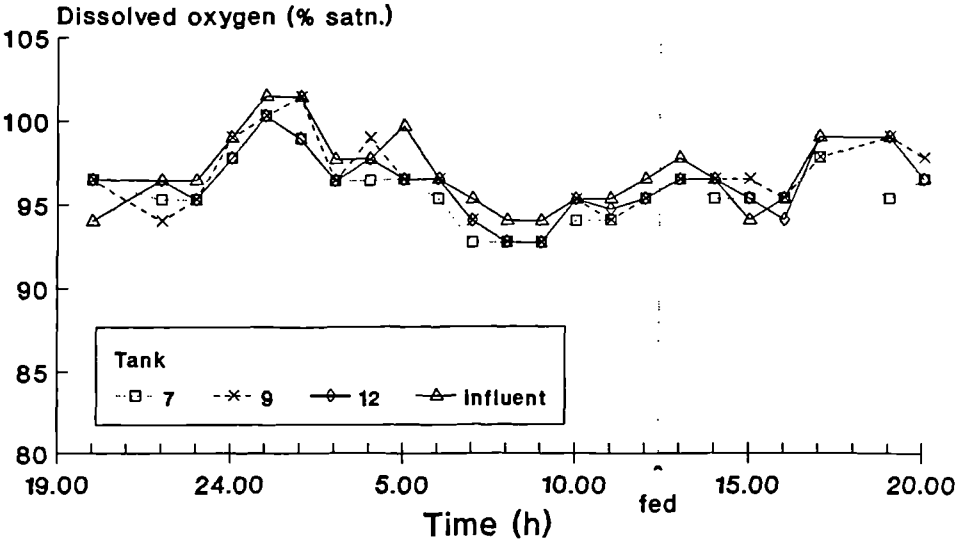
DO concentration fluctuated between 7.2 and 8.2 mg l^{-1}, corresponding to a percentage saturation fluctuation between 90.2 and 101.5 % with a mean of 95.8 % (Figures 8.26 and 8.27). The maximum saturation detected within a tank at any time was 101.5 %. Water in all the tanks examined followed a similar pattern of DO fluctuation, which was also similar to fluctuations in the influent water DO. Maximum DO concentrations occurred at approximately 02.00 and 19.00 h. Low DO values, relative to the rest of the sample period, occurred between approximately 07.00 and 16.00 h. A fall in DO occurred within 1.5 - 2.5 h after feeding in all tanks and influent water. Water in all tanks and influent water exhibited a marked increase in DO simultaneously, between 3.5 and 4.5 h after feeding and between 2.5 and 3.5 h after cleaning. Figures 8.26 and 8.27 clearly demonstrate a cyclical change in DO, with an amplitude of approximately 10 - 12 % saturation and a period of 24 h. Initial and

Figure 8.26: DO concentration changes during a 24 h period - same residence time tanks



Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 18cm. Influent = head tank

Figure 8.27 DO concentration changes during a 24 h period - same flow rate tanks



Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 18cm. Influent = head tank

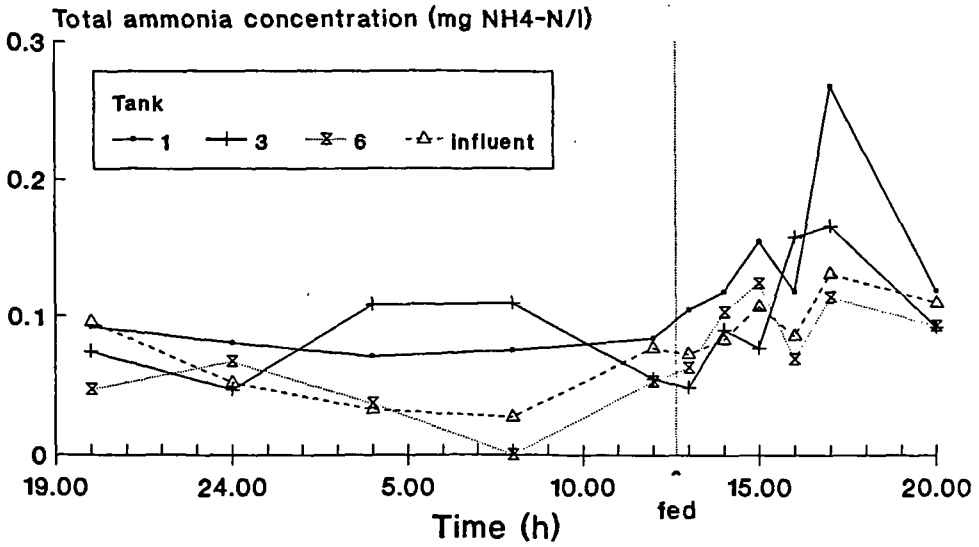
final DO values were not the same, so the estimate of the cycle period is approximate.

Influent water DO was usually greater than the treatment tank water. Tank 1 (3 cm depth) contained water with the lowest, or equal lowest, DO of any tank with the same residence time. Tank 7 (3 cm depth) also contained water with the lowest DO, in 19 of the 23 samples. DO differences between 9 and 18 cm deep tanks were less obvious. Tank 6 (18 cm) however contained water with a higher than, or equal DO saturation, to tank 3 (9 cm) in 20 of the 23 occasions, though values were equal in 9 of these samples. The shallowest tanks tested (3 cm) clearly contained water with a lower DO throughout the sample period, than the other deeper tanks tested, at either the same residence time or flow rate.

Total ammonia

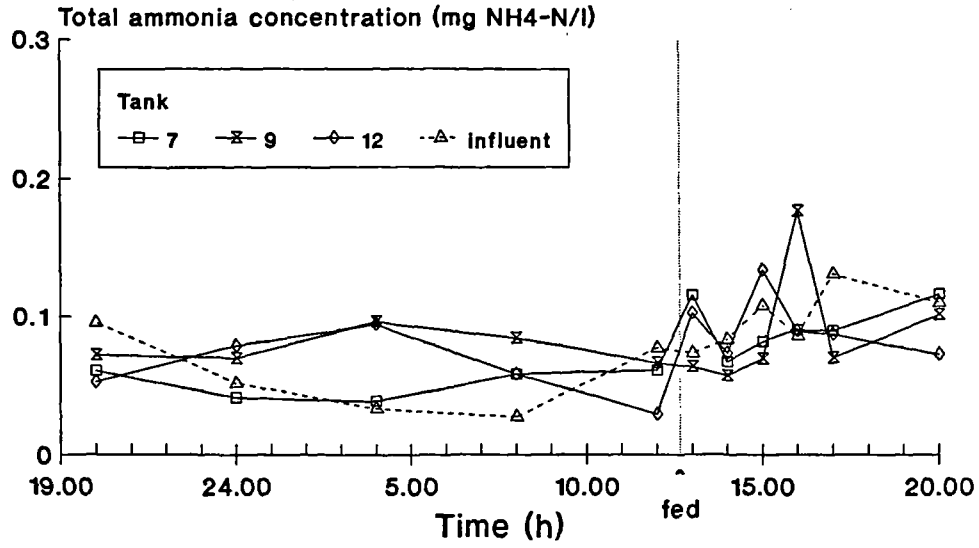
NH_4-N concentrations ranged between 0.027-0.268 mg NH_4-N l^{-1}, a 10 fold variation. Two distinct periods of NH_4-N concentration fluctuations through the 24 h sample period were evident (Figures 8.28 and 8.29). A period of relatively constant NH_4-N concentrations, from the first sample time until feeding (20.00 - 12.30 h), was followed by a more variable period after feeding until the final sample time (12.30 - 20.00 h). NH_4-N concentrations increased from overnight levels, to peak approximately 2.5 - 4.5 h after feeding. This trend was partially masked by

Figure 8.28: Total ammonia concentration changes during a 24 h period - same residence time tanks



Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 18cm, Influent = head tank

Figure 8.29: Total ammonia concentration changes during a 24 h period - same flow rate tanks



Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 18cm, Influent = head tank

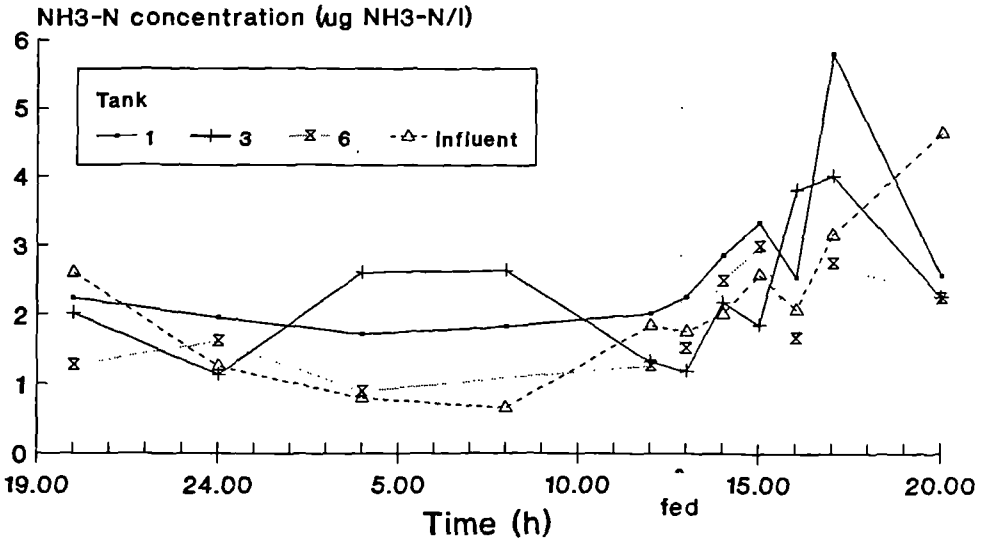
the increased fluctuations. After the peaks, a rapid decrease in concentration was observed.

Before feeding, no clear association between NH_4-N concentration and depth was apparent. Treatment tank and influent water NH_4-N concentrations were similar. After feeding, the NH_4-N concentration in tank 1 (3 cm water depth, constant residence time) increased more quickly and to a higher level than in any other tank. The maximum concentrations attained, and the time after feeding of the peaks, appear related to the tank depth. Despite having the same residence time as tanks 3 and 6, tank 1 exhibited a maximum NH_4-N concentration which was 3 times the overnight value, 4.5 h after feeding. Tank 3 (9 cm depth) peaked to 2.5 times the overnight level, between 2.5 and 3.5 h after feeding, while tank 6 (18 cm) peaked to twice the overnight concentration, 2.5 h after feeding. This trend was not distinguished in tanks with the same flow rate. The flow in tank 1, at 0.57 l min^{-1}, was the lowest of any of the tanks. Ammonia concentrations in the deepest tanks, 6 and 12, were with one exception, the lowest of those tanks sampled on or after 16.00 h, ie. 3.5 h after feeding.

Unionized ammonia

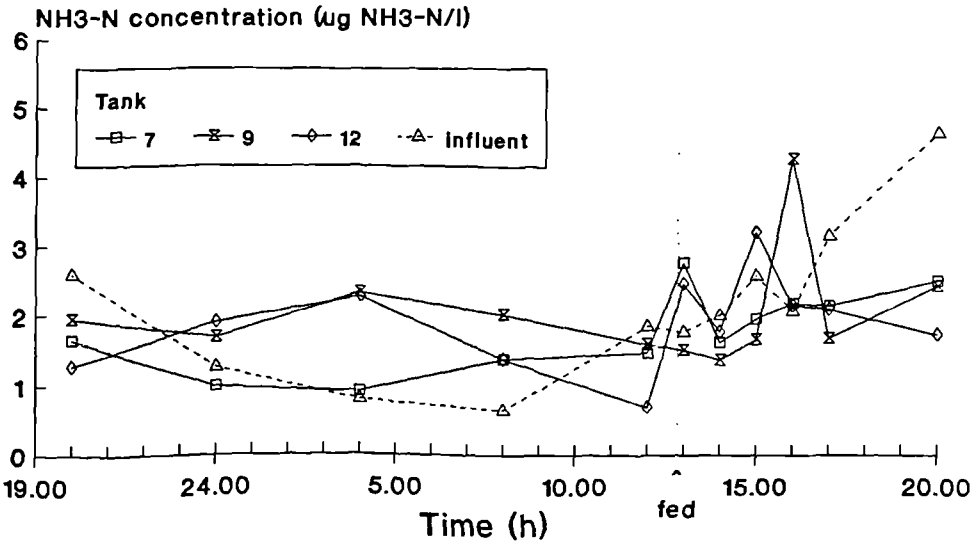
NH_3-N concentrations varied between 0.65 - 5.79 μg NH_3-N l^{-1} (Figures 8.30 and 8.31). The pattern of fluctuations was similar to those of corresponding ammonia

Figure 8.30: Unionized ammonia concentration changes during a 24 h period - same residence time tanks



Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 18cm, influent = head tank

Figure 8.31: Unionized ammonia concentration changes during a 24 h period - same flow rate tanks



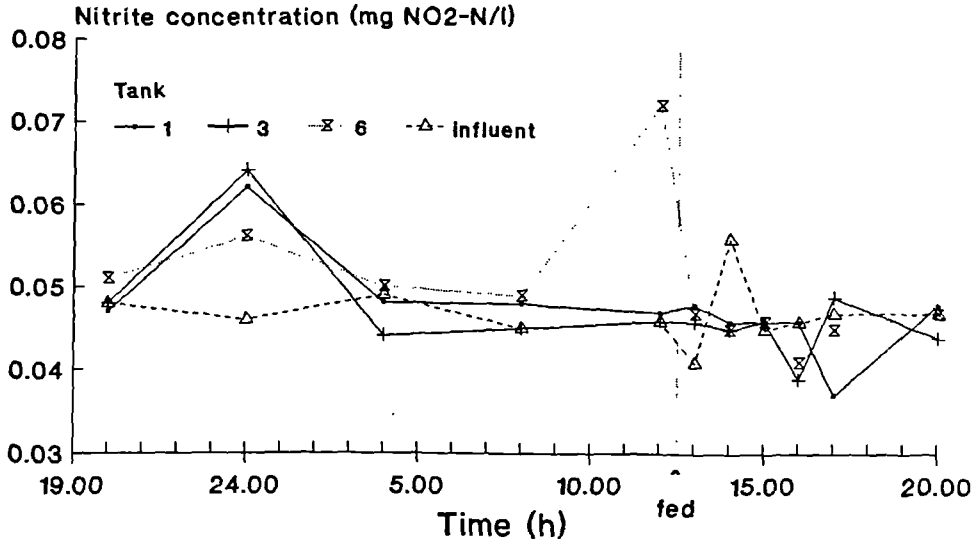
Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 18cm, influent = head tank

concentrations. A period of relatively stable NH_3-N concentrations occurred between the start of sampling and feeding the fish. After feeding, concentrations in the water of all tanks tested and the influent water rose to a maximum. Tank NH_3-N concentration reached maximal levels between 2.5 - 4.5 h after feeding, although the influent water NH_3-N concentration continued to rise to the end of the sampling period. Figure 8.30 shows a clear influence of water depth on maximum NH_3-N concentration in tanks with the same residence time. The shallowest tank 1 (3 cm depth) contained water with the highest concentration of 5.79 ug NH_3-N l^{-1}. Tank 6 (18 cm depth) contained the lowest maximum NH_3-N concentrations of 2.76 ug NH_3-N l^{-1}. Tank 3 (9 cm) was intermediate. Influent water concentrations were usually lower than tank values, throughout the survey. Water in tanks with the same flow rate did not follow this pattern: no recognizable influence of treatment on NH_3-N levels was evident.

Nitrite

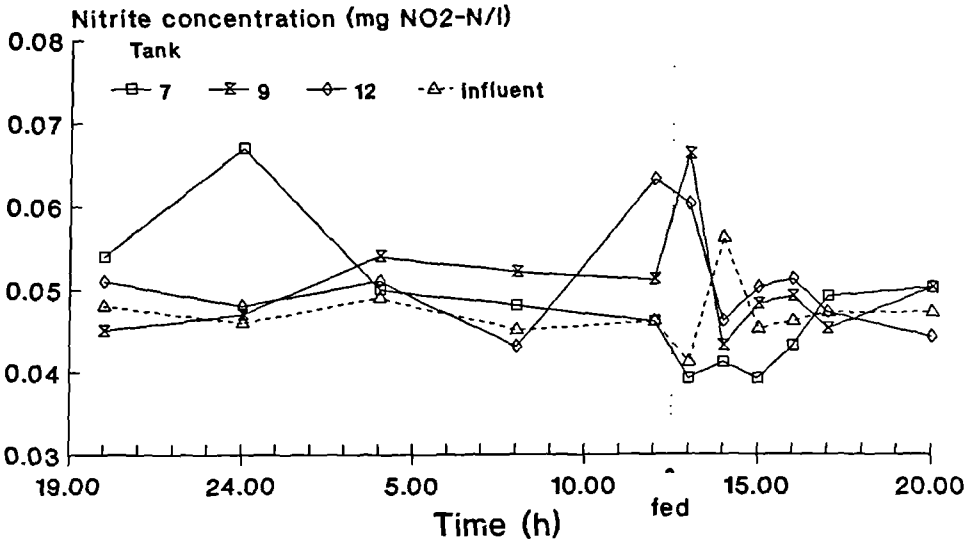
NO_2-N concentrations varied between 0.037 and 0.072 mg NO_2-N l^{-1} during the 24 h sample period (Figures 8.32 and 8.33). Fluctuations throughout the period were considerable so trends with respect to time or depth were not clear. Influent NO_2-N concentration appeared to peak 1.5 h after feeding, which was 21 h after the associated influent water NH_4-N concentration peak that was expected to have occurred the previous day. NO_2-N

Figure 8.32: Nitrite concentration changes during a 24 h period - same residence time tanks



Water depths: tank 1 = 3cm, tank 3 = 9cm
tank 6 = 18cm, influent = head tank

Figure 8.33: Nitrite concentration changes during a 24 h period - same flow rate tanks



Water depths: tank 7 = 3cm, tank 9 = 9cm
tank 12 = 18cm, influent = head tank

concentrations in tanks 1, 3, 6 and 7 peaked at 24.00 h, 11.5 h after feeding.

Some influence of depth on the magnitude of the peaks at 24.00 h was evident. Influent NO_2-N concentration was lower than any of the tanks at the time of the peak; the order of the water depth in the tanks, in decreasing NO_2-N concentration was, 3, 9 ,3, 18, 9, 18 cm, suggesting that a marginally higher maximum concentration of NO_2-N occurred in shallower tanks.

8.4.11 4 h water quality fluctuations

5 water samples obtained hourly between 12.00-16.00 h were taken on day 69, to examine further the expected critical period for water quality, following feeding at 12.30 h. Tanks 1, 6, 7 and 12, the extremes of depth, were sampled. The main results are shown in Table 8.12.

Temperature

Temperature fluctuations were less than +/- 0.2 ^{o} from 17 ^{o}C. This was on the limit of definition, so variations may then have been due to measurement error. Temperature was therefore considered as being an approximately constant 17 ^{o}C, which was 0.6 ^{o}C higher than the highest value attained during the earlier 24 h sample period (day 24 - 25).

Table 8.12: 4 h water quality results

Time	Location	pH	DO (% satn.)	mg NH_4-N l^{-1}	µg NH_3-N l^{-1}	mg NO_2-N l^{-1}
12.00	1	7.78	86.9	0.098	1.29	0.074
	6	7.86	90.8	0.108	1.81	0.067
	7	7.85	90.8	-	-	-
	12	7.86	89.5	0.063	1.06	0.047
	inf	7.82	93.3	0.090	1.19	0.066
13.00	1	7.78	80.5	0.164	2.16	0.066
	6	7.85	88.0	0.157	2.64	0.052
	7	7.85	88.0	0.084	1.41	0.070
	12	7.69	86.9	0.099	1.04	0.066
	inf	7.85	90.8	0.111	1.64	0.053
14.00	1	7.80	84.4	0.136	1.80	0.073
	6	7.84	89.5	0.093	1.38	0.068
	7	7.83	88.0	0.101	1.49	0.068
	12	7.83	89.5	0.092	1.36	0.048
	inf	7.84	90.8	0.092	1.36	0.069
15.00	1	7.77	86.9	0.150	1.77	0.067
	6	7.82	88.0	0.089	1.17	0.113
	7	7.81	90.8	0.096	1.27	0.067
	12	7.83	88.0	0.136	2.01	0.056
	inf	7.83	92.0	0.093	1.38	0.065
16.00	1	7.61	86.9	0.114	0.96	0.077
	6	7.82	90.8	0.113	1.49	0.071
	7	7.80	89.5	0.119	1.57	0.069
	12	7.82	86.9	0.130	1.72	0.057
	inf	7.82	90.8	0.127	1.68	0.061

Temperature = 17.0 ^{o}C Salinity = 35.1 ppt
Location = tank number or influent

pH

Tank water pH fluctuated within the range 7.61 - 7.86 This range was lower than pH values measured during the 24 h investigation 45 days previously. The pH of the water in all the tanks was lower than the influent water pH, throughout the 4 h period. A small but constant decrease in pH of approximately 0.02 over 4 h in all tanks and influent, except tank 1, was evident and was similar to a background decrease detected during the 24 h sample

period. The pH of water in tank 1 (3 cm depth) was consistently lower than both the influent water and tank 6 (18 cm depth) pH, at the same residence time. This was a similar situation to that determined from the 24 h sample period results. The pH of tank 1 fell markedly 2.5 h after feeding and continued to fall until the end of the sample period. A similar fall during the 24 h sample period was detected within 30 min of feeding. A decrease in pH in tank 12 (18 cm depth), 30 min after feeding, was similar to a decrease determined during the 24 h survey, but during the 4 h survey the pH increased before the following sample 1 h later.

Salinity

Salinity was measured as 35.1 ppt throughout the sample period, with no detectable fluctuations. The salinity was the same as that measured during the 24 survey.

Dissolved oxygen

The DO concentrations and saturations of water in tank 1 were, in every sample, lower than or equal to, any other tank or influent. The lowest percentage saturation value determined from tank 1 was 80.5 %. Tank 1 had the lowest flow rate of any tank, but the same residence time as tank 6 which consistently contained water with a higher DO concentration. Concentrations in the other tanks and

influent were generally lower than values determined during the 24 h survey. A period of lowered DO levels after feeding, shown in Figures 8.26 and 8.27, was not observed in the 4 h survey results, except in tank 1, in which a fall was apparent 30 min after feeding. The DO concentration of influent water was constantly higher than in water in any of the tanks.

Total ammonia

A peak in NH_4-N concentrations occurred much sooner after feeding during the 4 h survey than the 24 h sample period. During the former period NH_4-N concentrations in tanks 1 and 6 rose to a peak 30 min after feeding. During the latter survey this peak did not occur until approximately 2.5 - 4.5 h after feeding. NH_4-N concentrations did not fall greatly after this peak. Concentrations in tank 7, 12 and the influent water gradually increased to the end of the sample period, so no peaks were detected at these three locations. The range of NH_4-N concentrations of between 0.063 and 0.157 mg NH_4-N l^{-1} was within the range detected during the 24 h survey (0.027 - 0.268 mg NH_4-N l^{-1}).

Unionized ammonia

NH_3-N concentrations varied between 0.96 and 2.64 ug NH_3-N l^{-1}, which was a similar range to that shown in the 24 h sample period results. The concentrations in

tanks with the same flow rate, tanks 1 and 6, peaked 30 min after feeding. NH_3-N concentrations in water in tank 12 peaked 2.5 h after feeding, while in tank 7 and the influent water, concentrations showed no pronounced peaks. No influence of depth on the size or timing of the peaks was observed.

Nitrite

NO_2-N concentrations, which were similar in range to those determined during the 24 h sample period, varied between 0.047 and 0.113 mg NO_2-N l^{-1}. Tank 1 (3 cm) contained water which had the highest NO_2-N concentration of any tank or influent during 3 of the 6 sample periods. No other effect of depth or feeding on NO_2-N concentration was observed.

8.4.12 Within-tank water quality

Water analysed to indicate water quality changes across the tanks was sampled at 11.30 h on day 4 as stated in section 8.3.6.

Temperature

The temperature of all samples was 16.2 °C.

pH

pH differences between sample points both within a tank and between different tanks were negligible, varying by no more than +/- 0.02 about the mode 8.17.

Salinity

The salinity of all samples was 35.5 ppt.

Total ammonia

NH_4-N concentrations during the within-tank survey ranged between 0.053 - 0.198 mg NH_4-N l^{-1} (Figure 8.34). These concentrations were generally higher than those during the 24 h sample period, and approximately double that of the equivalent 11.30 h value shown in the 24 h sample period results. In tanks with the same residence time, the NH_4-N concentration in the centre, influent and effluent of the shallowest tanks were similar. In deeper tanks effluent and influent concentration appear similar, but tank centre concentrations were lower. NH_4-N concentrations of water samples from tanks with the same flow rate were similar to each other and the influent water.

Unionized ammonia

Salinity and temperature varied little between

samples, so NH_3-N concentrations followed a similar pattern to the NH_4-N results ranging between 1.44-5.37 μg NH_3-N l^{-1} (Figure 8.35).

Nitrite

NO_2-N concentrations varied between 0.041-0.50 mg NO_2-N l^{-1} (Figure 8.36). The influent NO_2-N concentrations of all tanks with the same residence time, was lower than or equal to the concentrations at the tank centre or effluent. No other influence of water depth, or position of the sample point, on NO_2-N concentration was observed.

8.4.13 Summary of main results

1. Survival: only 1 fish died during the experiment.
2. Weight: initial and final tank mean weights were not correlated.
3. Weight: during days 0 - 56, malpigmented fish weighed significantly more than pigmented fish only in tank 5 (15 cm water depth).
4. Weight: no other significant associations were found between the distribution of fish weights and either pigmentation type, water depth, or flow type.
5. Length: malpigmented fish were only significantly longer than pigmented fish in tank 5 (15 cm depth) during days 0 - 56.

Figure 8.34: Total ammonia concentration changes across the treatment tanks

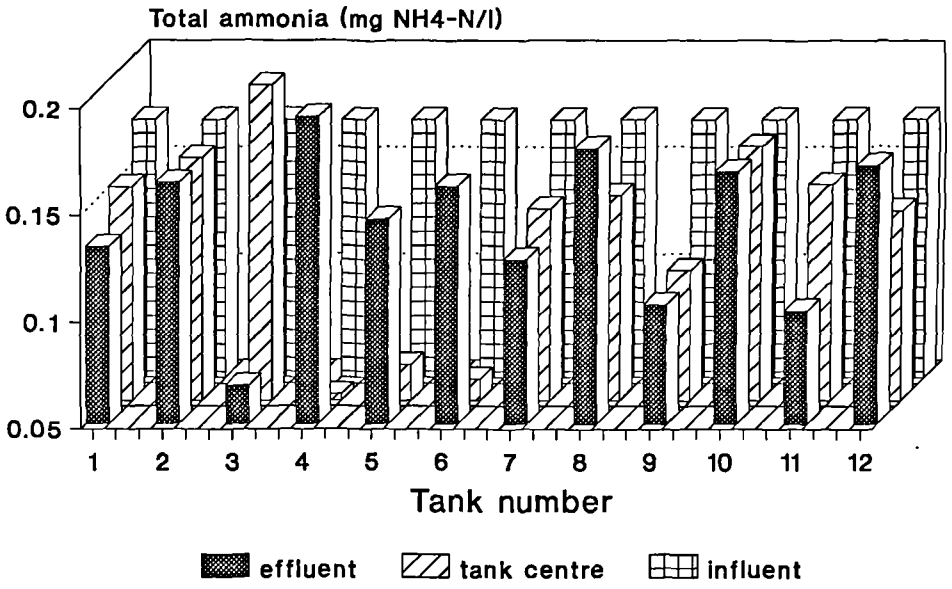


Figure 8.35: Unionized ammonia conc. changes across the treatment tanks

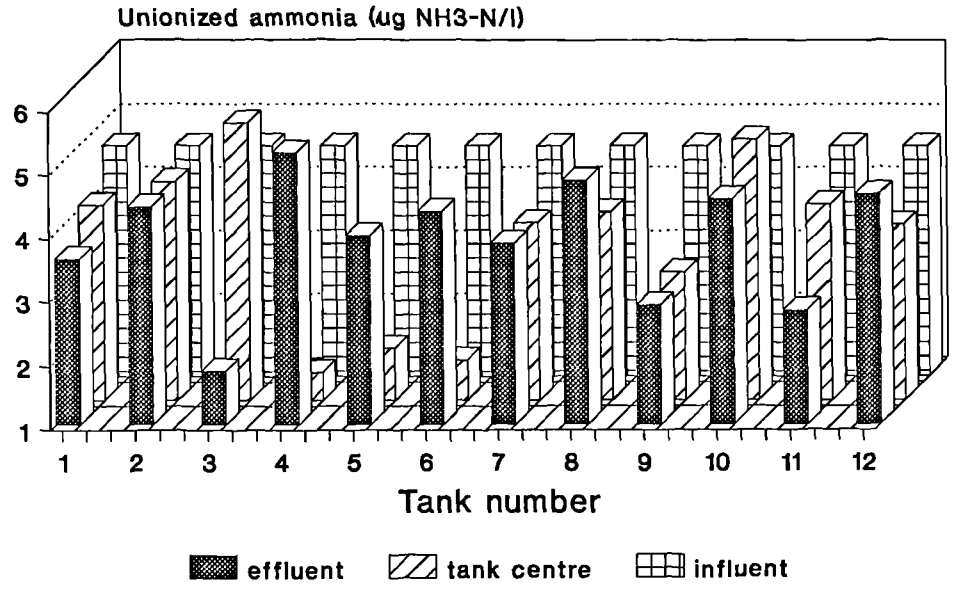
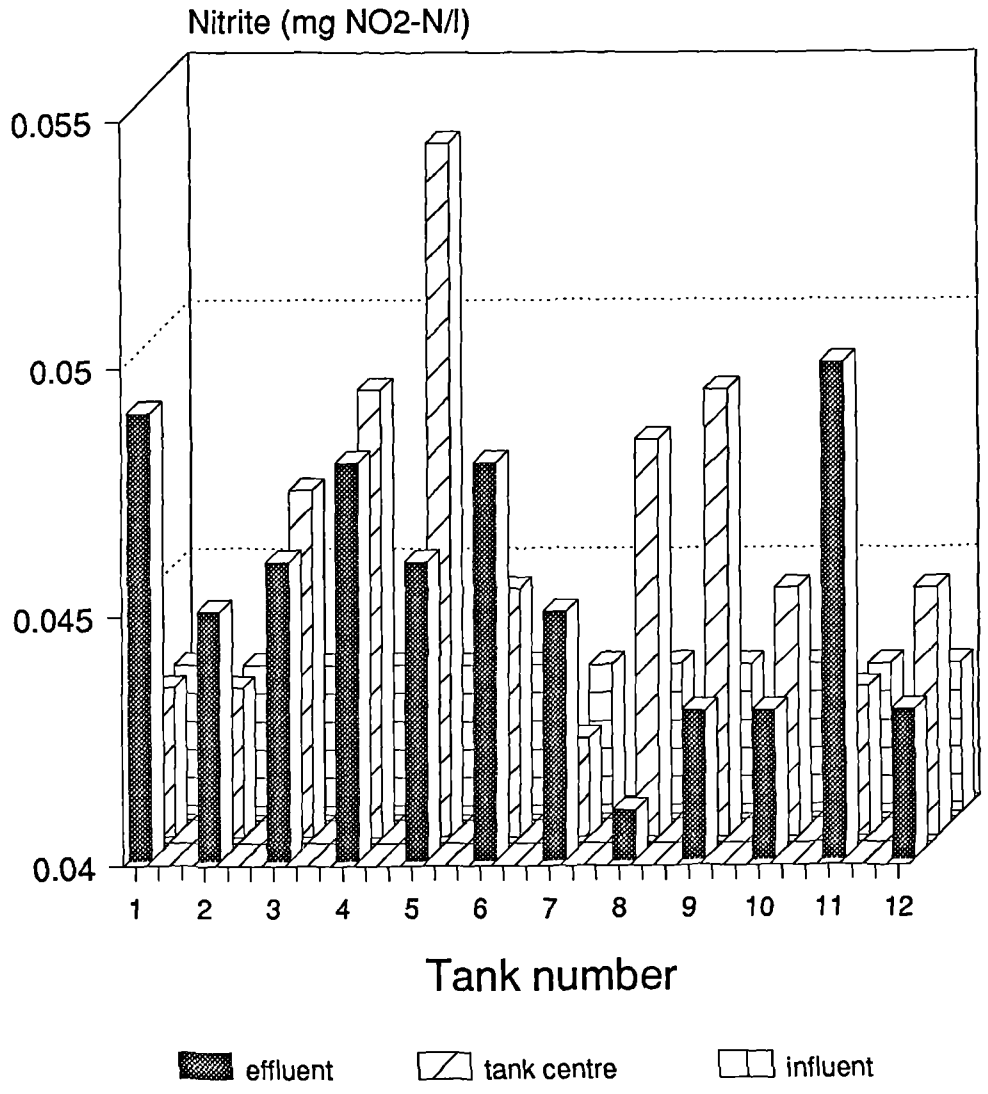


Figure 8.36: Nitrite concentration changes across the treatment tanks



6. Length: no other significant associations were found, between the distribution of fish lengths and pigmentation type, water depth, or flow type.
7. CF: increased from an overall mean of 1.71 at day 0, to 1.80 at day 70.
8. CF: all tank mean CFs increased between days 0 - 70.
9. CF: tank mean CF in 11 of the 12 tanks decreased between days 0 - 14.
10. CF: final CF was similar irrespective of the initial value.
11. CF: neither individual fish or tank mean CF was found to be significantly influenced by pigmentation type, flow type, or water depth within the range 3 - 18 cm.
12. G_W: overall mean G_W during the experiment (tanks 1-12, days 0 - 70) was 0.83 % day^{-1}.
13. G_W: during the period 28 - 42 days malpigmented fish grew significantly faster (0.32 % day^{-1}) than pigmented fish.
14. G_W: the G_W of pigmented fish increased significantly with increased depth during the period 42 - 56 days as described by the equation $y=0.598+0.047x$.
15. G_W: the G_W of pigmented fish increased significantly with increased depth during the period 0 - 70 days as described by the equation $y=0.581+0.020x$.
16. G_W: the G_W of fish in tanks with the same flow rate increased significantly with increased depth during the period 0 - 14 days, as described by the equation: $y=0.267+0.030x$ ($r=0.609$, d.f.=10, $p<0.05$).

17. G_w: the G_w of fish in tanks with the same flow rate increased significantly with increased depth during the period 42 - 56 days, as described by the equation: $y=0.779+0.038x$ ($r=0.613$, d.f.=10, $p<0.05$).
18. G_w: the G_ws of fish in tanks with the same depth, but with either flow rate or residence time controlled, were not significantly different.
19. G_w: the mean G_w of all fish increased significantly with increased depth during the period 42 - 56 days, as described by the equation: $y=0.744+0.032x$ ($r=0.524$, d.f.= 22, $p<0.01$).
20. Vu: increased significantly in most tanks throughout the experiment.
21. Vu: no influence of depth on Vu at any time during the experiment was determined.
22. Food consumption: increased throughout the experiment from an overall mean of 1.27 - 2.82 % of body weight day^{-1} (wet/live weight) on days 14 and 56 respectively.
23. FCR: decreased during the middle of the experiment.
24. FCR: values of growing fish varied within the range 1.15 - 11.58.
25. FCR: improved significantly with increased water depth during the periods 14 - 27 and 42 - 55 days. An increase in water depth produced an approximate decrease in FCR of 0.45 (dry feed/live weight) during both periods.
26. System water quality:

    temperature = 15.2 - 17.8 ^{o}C;

pH = 7.86 - 8.16;

salinity = 34.6 - 35.8 ppt;

DO = 7.1 - 8.6 mg l^{-1} or 91.4 - 109.9 % saturation

27. 24 h temperature: was not significantly influenced by depth.

28. 24 h pH: a pronounced fall in pH in the treatment tanks was observed after feeding.

29. 24 h pH: decreased most quickly and furthest after feeding in the two shallowest, 3 cm deep, tanks.

30. 24 h DO: DO decreased in all tanks and influent water 1.5 - 2.5 h after feeding.

31. 24 h DO: the 2 shallowest tanks appeared to contain water with a generally lower DO compared with other tanks and the influent water.

32. 24 h total ammonia: concentrations varied between 0.027 - 0.268 mg NH_4-N l^{-1}.

33. 24 h total ammonia: concentration peaks occurred 2.5 - 4.5 h after feeding, followed by a rapid decrease in concentration to an overnight baseline level.

34. 24 h total ammonia: concentration peaks were greater and occurred sooner after feeding in shallower tanks with the same residence time.

35. 24 h unionized ammonia: varied between 0.65-5.79 ug NH_3-N l^{-1}.

36. 24 h unionized ammonia: appeared related to depth in constant residence time tanks. Lower levels occurred in the deeper tanks.

37. 24 h nitrite: concentration varied between 0.037-0.072 mg NO_2-N l^{-1}.

38. 24 h nitrite: concentration may have been generally lower in shallower tanks irrespective of flow type.

39. Within-tank water quality: no influence of position within the tanks, on temperature, salinity and pH was found.

40. Within-tank nitrite: influent NO_2-N concentrations were less than or equal to the tank centre and effluent concentrations.

8.5 DISCUSSION

8.5.1 Survival

Heap and Thorpe (1987) growing similar sized turbot to those in this study achieved survival rates of 97.3 % over a 118 day period, which they considered high. A survival rate of 99.2 % during the 70 day period of this experiment may then be considered good. Such a high value indicates that stress, an accepted precursor to death, was not great during the experiment. Five aspects of this experiment may cause sufficient stress to produce death: genetic parameters eg. fitness and adaptability; environmental parameters, eg. water quality, water depth; intraspecific interaction, eg. aggression; physical parameters, eg. handling; and nutrition, eg. pellet availability, quantity and nutritional quality. The only animal that died, did so near the end of the experiment, which suggests that it was some aspect of the experimental regime which caused its demise, rather than being an initially unfit animal. Overall mortality was low, so the recirculation system and treatments used can be considered at least adequate to maintain juvenile turbot. Having survived weaning, the viability of juvenile turbot is good (Smith, 1979) and mortality rate decreases with age during a period of 20 months (Purdom *et al.*, 1972). It was therefore expected that conditions would have to be bad to produce large scale mortalities during this experiment.

8.5.2 Fish weight and length

Fish weight and length fluctuations with time in each tank appeared similar. Equivalent increases in weight and length occurred in many tanks. This implies that if changes in these parameters were similar in different tanks, the final weight or length achieved was due to the initial weight or length distribution. Figures 8.1 - 8.4. appear to confirm this suggestion, however statistical analysis indicated that final weight or length was not a consequence of initial weight or length. Two reasons explain this apparent paradox. Firstly, confidence intervals of the mean values, which were, for clarity, not shown in Figures 8.1 - 8.4 indicated that, throughout the experiment, the weight and length distributions of fish in any tank were not significantly different to the distributions in any other tank. This conclusion was confirmed by ANOVA tests. Whilst mean weights or lengths appeared different, actual differences were small, due to the range of the distributions. Secondly, the range of mean weights and lengths at the beginning of the experiment, relative to the overall change in these parameters, was small, so minor differences in weight or length change greatly influenced the ranking of the tank mean values.

With one exception, the lack of significant differences in weight and length distributions at the beginning of the experiment indicates that random

assignment of fish to each of the tanks at day 0 produced the homogeneous distribution of weights and lengths desired, so this possible method of biasing the results was minimised.

A comparison between the weight distributions of pigmented and malpigmented fish in the same tank indicated that in only one tank was there any significant difference in the distributions. The difference was evident at day 56, but a significant weight difference also occurred at the beginning of the experiment (day 0). A similar comparison between the length distributions of pigmented and malpigmented fish indicated a significant difference in distribution during days 0 - 56 in the same tank 5. It is therefore likely that the difference in mean weight and length between the pigmentation groups in tank 5 was an artifact of the initial random assignment of fish to each tank, rather than a consequence of pigmentation. Though the initial weight and length distributions of fish within a tank were considered random, as described above, distribution within pigmentation type was not random. Such non-random distributions always have a small probability of occurring. Assuming this difference was a chance occurrence resulting from the initial assignment of fish to the tanks, it was concluded that pigmentation type did not influence either fish weight or length distribution during this experiment.

Results of investigations into the influence of water

depth on fish mean weight and length, allowing for the factor of flow type, ie. residence time or flow rate, indicated that no significant associations occurred.

Results of investigations using combined flow type weight or length data, which increased the sample size for the analyses, indicated that the distribution of these parameters was not influenced by water depth. The results show that the distribution of weights or lengths was statistically similar within the range of depths tested. The conclusion drawn from the previous two statements is that a reduction in water depth, even to as shallow a depth as 3 cm did not therefore influence fish weight or length distribution during the 70 day period of this experiment. It is however possible that the rearing period was not long enough to produce large differences due to treatment, because the G_W values were not great. The range of G_W values was 0.84 - 1.32 % day_{-1} and the overall mean weight was 5.72 g at day 0. Fish growing at these two extremes of the G_W range from the same initial weight of 5.72 g would be expected to differ by only 50 % after 90 days.

The similarity between the weight and length results also indicates that, while weight was more variable in the short term than length, due to differences in food consumption, digestion time etc., this parameter was at least as suitable a measure of fish performance, under different treatments, as fish length.

8.5.3 Condition factor

CF in this experiment was used to compare the fitness of fish under different treatments, ie. water depth, flow rate and residence time, and to compare the fitness of fish in different groups, ie. pigmentation type. Comparison of CF in this way comes under the definition of one of its most effective uses, as described by Weatherley (1972) and Knights (1982). Weight is likely to decrease more rapidly than length during periods of stress, as described by Dave et al. (1975), as reserves of energy are used and not replaced. A decrease in the value of CF is then an indication of worsening fitness.

Tank mean CF increased in all the treatment tanks, indicating an improvement in fish condition as the experiment progressed. The general nature of the increase suggests that some overall factor was responsible, rather than specific treatments. Results of the statistical analyses, which failed to find any associations between CF and treatments, confirmed this hypothesis. Several explanations may account for the small increase in fitness of fish in most of the tanks. All turbot of this size may increase in CF as they grow, though no published data verifying this has been found. Some general aspect of the environment may have been responsible, eg. nutrition or a water quality parameter occurring in all tanks, such as temperature or salinity (to be discussed below).

It may be expected that fitter fish, in terms of a higher initial CF would be most likely to continue to be fitter. This was not however the case in this study. Fish adapted to circumstances prior to this experiment may not have been suited to the particular conditions of this experiment. Hence final and initial CFs were not significantly correlated. Also the decline in CF of fish in the majority of the tanks at the beginning of the experiment suggests that the factor which influenced fish fitness did not affect CF during this period, probably because a period of acclimation to the new experimental conditions was not allowed.

Results of analyses comparing the CFs of pigmented and malpigmented fish were contradictory and significant differences were fragmented. It is likely that differences in CF between the two pigmentation groups were chance occurrences.

The lack of a significant influence of depth on CF indicated that either: depth does not influence CF; or the depth range tested was not extreme enough to influence CF; or methods employed to determine CF differences were inadequate to define differences due to depth. The latter explanation was unlikely to be responsible because CF differences, with time or between fish, of approximately 0.03 could be resolved. Rosenthal et al. (1982) has suggested a link between fish condition and poor mixing in tanks, and Burley and Klapsis (1985) have shown that

mixing is influenced by water depth. It is likely therefore that depth does influence fish condition, so the first explanation is unlikely to be correct. It is probable that the range of treatments adopted in this experiment was insufficient to produce the poor water quality experienced by Rosenthal et al. (1982). This will be discussed in the following sections, but results indicate that water quality was rarely critical and that differences between treatments were either small or occurred only for short periods.

8.5.4 Specific growth rate

Two distinct periods of growth were apparent during the experiment. Between days 0 - 42, G_W in most tanks decreased slightly or remained approximately similar to the initial G_W of 0.5 - 1 % day^{-1}. Between days 42 - 70, G_W increased to an overall mean of 1.43 % day^{-1}. The overall G_W, between days 0 - 70, was however only 0.83 % day^{-1}.

Before discussing the relative changes in G_W it is important to put these values into context. G_Ws achieved in commercial turbot farms are not well advertised for business reasons, but some data are available. Strict comparisons of G_Ws between studies are not reliable because differences in treatment, environment and the age, size and life stage of turbot will influence G_W. However general comparisons are possible.

G_W values of 3 % day^{-1} or more have been reported by Person Le-Ruyet *et al.* (1982), who achieved 3.4 % day^{-1} from 12 g fish and 2.08 - 4.80 % day^{-1} from 23 g turbot. Bromley (1980b) grew 15 g turbot at 1.6 - 3.0 % day^{-1}.

G_W values between approximately 2.0 - 3.0 % day^{-1} have been reported by workers including: Caceres-Martinez *et al.* (1984) who grew 10 g turbot at 2.33 - 2.51 % day^{-1}; and Poxton *et al.* (1982) who grew 3 - 370 g turbot at approximately 2 % day^{-1}.

Many workers have achieved between 1.0 - 2.0 % day^{-1} including Purdom *et al.* (1972) who grew 17, 23 and 40 g turbot at 1.2, 1.8 and 1.7 % day^{-1} and Bowers and Landless (1969) who, feeding to satiation, achieved highly variable G_W values of 0.95 - 2.48 % day^{-1} in 16 - 27 g turbot. Adron *et al.* (1976) managed 1.3 % day^{-1} in 10 - 41 g fish, and Adron *et al.* (1978) managed a similar 1.2 - 1.3 % day^{-1} in 16 - 19 g fish. Using a similar recirculation system to that employed in this experiment and turbot from the same supplier, Lake (pers. comm.) achieved G_Ws of 1.59 - 1.78 % day^{-1} from 17 - 83 g turbot.

G_Ws of less than 1.0 % day^{-1} have been reported in studies where some aspect of the conditions or treatment was considered sub-optimal. Calcedo Juanes (1988) attained G_Ws of -0.26 - 1.01 % day^{-1} in 7 - 42 g control fish, compared with 0.14 - 1.00 % day^{-1} from 8 - 31 g turbot fed on a silage diet. Feeding initially 17 - 27 g

turbot on a restricted diet at 17 ^{o}C Bowers and Landless (1969) achieved G_ws of 0.04 - 0.69 % day^{-1}. Similarly, Bromley (1980b) feeding only a maintenance diet obtained G_ws of 0.04 - 1.62 % day^{-1}. In a recent review of turbot growth rates, Calcedo Juanes (1988) considered that under conditions of good management, juvenile turbot should grow at a G_w of approximately 2 % day^{-1}. The majority of the authors cited above however obtained G_w values in excess of 1.7 % day^{-1}, so this latter figure would seem a more realistic target. This value does not of course allow for differences in environmental conditions, particularly temperature, and so serves only as an estimate of what may be considered, "adequate growth".

Assuming a nominal G_w of 1.7 % day^{-1}, it can be seen that the majority of G_ws achieved in this study were considerably less than this value. Only in tanks 4 and 5 (12 and 15 cm depth at the same residence time) during the final growth period, did tank mean G_ws exceed this value. Indeed during days 0 - 42 the turbot in this study were maintaining a G_w expected from fish on only a maintenance diet.

G_w values in this experiment were calculated from the mean change in weight of groups of 5 or 10 fish. Individual fish G_ws were likely to be more variable than the mean values, so certain fish may have achieved adequate or high growth rates, but were not distinguished due to the method of calculation. Tank and pigmentation

type mean G_W values do however give a general indication of the situation.

Clearly most fish were growing more slowly than 1.7 % day^{-1}. Some form of stress, which was not serious enough to cause mortalities, but also influenced condition factor, was occurring in all the tanks, irrespective of experimental treatment. This stress appeared to decrease, or fish were acclimatizing to it, towards the end of the experiment.

Several explanations may account for this poor fish performance during the experiment: poor water quality, handling, initially unfit fish, feeding frequency and feed quality. These factors will be discussed as they arise in the following sections of this chapter.

Such low growth rates, indicating poor performance, cast doubts on the ability of this experiment to define differences in fitness due to artificially altered treatment, ie. water depth. It is however possible that the influence of treatment on the biological indicators of fish fitness and performance may be more evident in stressed fish. Unfit fish may be more susceptible to further stress because they would be less able to tolerate the situation. Fully fit fish would be expected to be more hardy and may continue to prosper in marginally adverse conditions. The possibility of resolving the

influence of depth on fish growth and fitness did exist, so a full investigation was continued.

Pigmented and malpigmented fish were shown to have similar G_Ws throughout the experiment. The significant difference in G_Ws which developed between the two groups during days 28 - 42 may have been due to chance, because during the growth periods immediately before and after this time, differences were small. Higher G_Ws of malpigmented turbot were however reported by Heap and Thorpe (1987) who described G_Ws of 1.45 and 2.17 % day^{-1} for pigmented and malpigmented turbot respectively, at 14 ^{o}C. They implied that malpigmented turbot were better suited to intensive culture than pigmented turbot. During their study no account was made of genetic differences between their pigmented and malpigmented stock. Their malpigmented fish may have grown better because the parents were fast growing rather than the consequences of malpigmentation. During the present study the genetic relationship between the fish was not known, but between days 28 - 42 G_Ws were generally at their lowest, so it is possible that under extreme conditions of stress or fitness, malpigmented fish were more hardy than pigmented fish. Evidence for this proposal is limited.

During two periods of the experiment (42 - 56 and 0-70 days) the G_W of pigmented fish increased significantly with increased depth. Similarly G_W of fish in tanks with the same flow rate, increased significantly with increased

depth during two periods (0 - 14 and 42 - 56 days). No significant correlations were found between growth and depth for the same group of fish, held in tanks with the same flow rate, during days 14 - 42. This suggests that the significant association between G_W and depth during days 42 - 56 may not merely have been an artifact of the previous significant result which occurred soon after the initial assignment of fish to the tanks. Though a significant influence of depth on G_W did not persist throughout the experiment these results do indicate that depth or a secondary parameter associated with depth, such as water quality, influenced the growth of turbot. A further significant correlation between depth and the G_W of all fish during the period 42 - 56 days confirms this suggestion. The similarity in the b values quantifying the association between G_W and depth is further evidence to suggest that the significant correlations, though not persistent, were unlikely to be purely the result of chance occurrences.

Several parameters associated with depth may have influenced G_W. Water quality may have been better in the deeper tanks. The behaviour of turbot may not have been suited to shallow water depths. The hydrodynamics in shallow tanks may have been inferior, or some other factor such as feeding may have been influenced by depth. These parameters will be discussed as they arise in following sections.

No significant associations occurred between water depth and G_W of fish in tanks with a variable flow rate, unless these fish were grouped with other fish. Therefore it was alteration of the residence time, coincident with depth, which was important in determining G_W. The reverse situation to that expected occurred: G_W decreased with decreased residence time, ie. increased flushing. This will be discussed further in the following water quality sections.

The significant association between the initial (days 0 - 14) and overall (days 0 - 70) G_Ws is evidence that initially faster growing fish grew better during the experiment, though this situation was not indicated by CF results. The initial performance may have been an artifact of the stock history, or an indication that the faster growing fish at the beginning of the experiment were better suited to the conditions of the experiment and therefore grew quicker. Care should be taken in the emphasis that is placed on this conclusion because final and overall G_Ws were not independent variables.

8.5.5 Coefficient of variance

Purdom (1974) and Jobling and Wandsvik (1983) have shown that coefficient of variance can be used as a measure of dominance hierarchies within a population. In many species of fish, an increase in the size range of the captive population will occur as hierarchies are

established, when large fish succeed and small fish fail to compete. Similarly stressors other than intra-specific aggression may also be expected to differentially affect individual fish.

G_w in this study was calculated from a mean weight change of 5 or 10 fish, so G_w variation about this mean was not known. V_u can be used to indicate the extent of variation in the distribution of fish weights. Purdom (1974) states that, unlike variance, V_u would not be expected to increase with fish weight, all other things being equal. V_u can be used as a measure of social interaction within a population, so it was only calculated to include all fish in a tank.

The V_u of fish in each tank at the beginning of the experiment was shown to be statistically similar. Assuming size range, rather than the inherent level of aggression of individual fish, is an indication of dominance hierarchies, the extent of the development of hierarchies in all 12 populations at the beginning of the experiment can be considered similar. Resulting differences in V_u between the tanks later in the experiment may therefore have been due to non-social stressors such as water depth. Though water depth is an environmental rather than social parameter, changes in water depth will change stocking density.

In 9 of the 12 tanks V_u increased significantly with

time. Fish weight also increased during the same period. Either V_u was not totally effective in allowing for inherent genetic increase in variation with mean weight, as proposed by Cripps (1983), or stress, causing a differential effect on large and small fish, was present in many of the tanks irrespective of treatment. The latter suggestion confirms the theory based on the decrease in G_w and increase in CF with time, which occurred in most treatment tanks. G_w was however shown to improve from the period 42 - 56 days until the end of the experiment, indicating a reduction in stress at this time, which was not evident in the V_u results. No influence of depth on V_u was found.

8.5.6 Food consumption and food conversion ratio

Food intake increased throughout the experiment coincident with the size of the fish, ie. weight and length, and not coincident with G_w. G_w showed an increase from approximately day 42 which was not evident in the food consumption results. This improvement in G_w, was therefore unlikely to be the result of an increase in food consumed, and so must have been due to some other factor.

Care must be taken when comparing FCE values quoted in this study with published data. Factors such as the composition of the diet, in particular the moisture content, method of calculation and environmental conditions, may influence the food conversion values

quoted. A general overview will however indicate whether the FCEs obtained in this study could be considered as good or poor.

Food conversions for juvenile turbot, all on a wet weight basis, of about 2 have been quoted by several authors including: Poxton et al. (1982) who managed 1.5-2.3 from 5 - 20 g turbot using semi-moist pellets similar to those used in this experiment; and Hull and Edwards (1976) who achieved an average of 2, also on moist pellets. WFA (1977) achieved a broad range of food conversion values between 0.97 - 2.92 from post-weaned turbot. Alternatively Smith (1979) achieved high values compared with these results, of 4.56 - 5.35 from fish with a weight of less than 4.7 g. Better conversions of 2 were however achieved, in the same study, with fish under 51.5 g. Calcedo Juanes (1988) achieved a broad range of conversion values within a recirculation system, dependant on the feed presented. FCR values of 1.42 - 1.93 were obtained from 6.9 - 41.6 g turbot on a control diet of moist pellets, the composition of which was adopted in this experiment, and 2.82 - 4.27 from 7.6 - 30.9 g turbot using a silage diet.

It can be seen then that FCR values of between approximately 1.4 - 2.0 are common in fish of a similar size to that used in this experiment, though other parameters may not have been strictly comparable. FCRs achieved in this study fall generally within this range,

though the variation was large. FCR was shown to decrease with increased water depth during two of the five growth periods.

8.5.7 Biological parameter conclusions

Overall the biological measures of turbot growth and fitness indicated that conditions were not ideal within the recirculation system as a whole, as shown by low G_w and CF and high FCR values. There was some evidence to suggest that the stress was being overcome or reduced during the second half of the experiment. Malpigmented fish occasionally grew faster than pigmented fish. The deeper tanks occasionally contained fish with a higher G_w and lower FCR, suggesting that environmental and stress conditions were better in the deeper tanks.

8.5.8 Temperature

Temperature requirements for the culture of turbot have been well documented, and most authors are in general agreement. Scherrer (1984) studied the influence of temperature, within the range 10 - 22 °C , on the growth of turbot and found no significant difference in growth within the range 16 - 22 °C. One of the earliest recommendations was given by Purdom *et al*. (1972) who

suggested that maximum growth rates occurred at 18-22 °C, while Waller (1985) stated that metabolism increased with increased temperature up to 17 - 22 °C. Many workers have achieved adequate or good turbot growth at the lower end of this scale, including Bromley (1980b) at 18 °C and Dickson (1977) who obtained the highest weight increases of his trials at 18 °C, compared with 15 and 21 °C. Lower temperatures than this were used by Deniel (1976) at 15 °C, Person-Le Ruyet et al. (1980) at 17+/-1 °C and Poxton and Allouse (1987) and Poxton et al. (1982) at 16 °C. Indeed Poxton and Allouse (1982) considered 16 °C optimal, with feeding rates maintained at a high level. Below this temperature the WFA (1977) found that the growth coefficient was directly related to temperature within the range 6 - 17.5 °C. Similarly Heap and Thorpe (1987) obtained higher growth rates in both pigmented and malpigmented fish at 14 °C, compared with 10 °C. Smith (1979) suggested that a minimum temperature range of 12 - 15 °C was required for adequate growth and survival for commercial culture.

Heap and Thorpe (1987) and Smith (1979) did not show any detrimental effects above a particular temperature, so it was likely that the optimal temperature for maximum growth was higher than their values, ie. 17.5 °C. Many of the authors quoted above adopted temperature regimes centred around approximately 16 - 18 °C suggesting that in their cases the lower end of the range suggested by Purdom (1972) was more suitable in practice. This is confirmed

by the results of Dickson (1977), so it seems likely that approximately 18 ^{o}C is optimum for the growth of turbot, and a reduction in growth down to a temperature of approximately 14 ^{o}C is acceptable. Poxton (in press) states that there is no single optimum temperature. Different temperatures will be optimal for different aspects of fish culture, such as growth, FCR, economic profit and life stages.

Based on these assumptions, measurements taken daily throughout this experiment indicated that temperature, which was less than 3 ^{o}C lower than the estimated optimum for growth, did not reach stressful levels. Growth may have been slower at the lower end of the temperature scale (15 ^{o}C) but fluctuations were large enough to ensure that the lowest temperatures did not occur for more than a day. All daily readings were taken at 11.30 h. Results of the investigation into temperature changes during a 24 h period indicated that temperature fluctuated diurnally. The fluctuation was found to be less than 1 ^{o}C during days 24 - 25. If this situation is considered representative of temperature fluctuations during other days of the experiment, which is not unlikely given the large volume of water in the experimental system and the high specific heat capacity of water, the minimum temperature at any time during the experiment was unlikely to be less than 14 ^{o}C.

Attention is drawn to the fact that the lowest

temperatures often occurred during a weekend, when the heating of the building which housed the recirculation system was shut down. The 24 h survey was carried out on a Sunday and Monday, so it is not surprising that a cycling in temperature was not apparent, because the building and the system water were heating up from a weekend low. A temperature survey during the middle of the week may have indicated a more cyclical pattern.

With residence times of only 3.09 - 18.54 min, significant differences in temperature between and within the tanks were not expected and did not occur.

8.5.9 pH

pH in culture systems has not been as well documented as many other water quality parameters, possibly because, all other parameters being equal, a broad range in pH can be tolerated by fish. Poxton and Allouse (1982) in a review of the subject suggested that mortalities can be expected in fish outside of the pH range 5 - 9.5 and recommended a narrower range of 6.5 - 8.5. The use of caution was suggested when applying this range to a practical situation because many factors complicate the condition. It is the secondary effects of pH on the toxicity of various substances to fish which are more important within this range. A fall in pH, which occurs during long term nitrification, reduces the proportion of toxic unionized ammonia and may increase the concentration

of carbon dioxide to toxic levels (Wickins, 1981). The toxic effects of pH are influenced by metals such as iron in the water (Ali, 1972), water hardness, size and age of the fish, acclimation and time of exposure (Poxton and Allouse, 1982).

pH, measured daily during the present study (7.86-8.16), was well within recommended ranges and in the absence of pollutants, other than low concentrations of total and unionized ammonia, was unlikely to have affected the fish.

Results of the investigation into 24 h fluctuations suggested that the overall trend in pH change shown in all tanks and the influent was the result of two interposing factors. A pronounced decrease in pH soon after feeding indicated that the recirculation system filtration was unable to cope with the influx of food and resulting increase in fish excretory products. The buffering capacity of the water and the filtration system reduced this decline after approximately 12 h following feeding. Imposed on this large decline there appears to have been a more gradual reduction in pH throughout the day. Honer et al. (1987a) appears to have experienced a similar situation.

Of those tanks in which pH was measured during a 24 h period, the two tanks with the lowest and fastest changing pH were the two shallowest tanks, irrespective of whether

flow rate or residence time was maintained the same. It is likely then that an adequate water volume is required to maintain pH even with relatively short residence times (ie. 3.09 min). The effect was less pronounced in tank 7 than tank 3, indicating that it may be possible to overcome the reduction in pH, due to inadequate water volume, by increasing the flow rate, so introducing a greater quantity of water with a higher buffering capacity and possibly higher pH. Honer et al. (1987a) and Rosenthal et al. (1981) found that the pH of the influent water was higher than that from the treatment tanks. This was not found in the present study but the buffering capacity ie. carbonate composition, may have been greater in the influent.

8.5.10 Salinity

Salinity was maintained at approximately 35.0 ppt +/- 0.8 (with one exception). The constituents were unlikely to be different to natural seawater because the recirculation system water was obtained from the sea and had not previously been used to culture animals. A depletion of elements was not therefore expected, so salinity or system water constituents were unlikely to stress the turbot. No short term changes in salinity, which may have produced osmotic shock in the fish, were detected. No differences in salinity within or between the tanks were detected.

8.5.11 Dissolved oxygen

DO levels tolerated by fish depend on species, life stage, temperature, carbon dioxide concentration (Doudoroff and Shumway, 1970), acclimation (Alabaster and Lloyd, 1980) and the length of exposure to sub-optimal conditions (Davison et al. (1959). Several studies have indicated the DO requirements of fish, including Doudoroff and Shumway (1970), EIFAC (1973), Davis (1975), Alabaster and Lloyd (1980), Wickins (1981) and Poxton and Allouse (1982).

Effects of critical levels of oxygen include the limitation of growth rate (Alabaster, 1973; Warren, 1971), reduction of food conversion efficiency (Adelman and Smith, 1970), reduction in first feeding (Brownell, 1980b) and death (EIFAC,1973). An estimate of the necessary DO levels is given by Davis (1975) who suggested that greater than 82 % saturation at 15 ^{o}C was required by marine nonanadromous fish to avoid stress symptoms.

DO in the recycle system, measured daily and throughout a 24 h period, did not fall below 90 % saturation, so a lack of oxygen did not stress the turbot. Many of the values did however indicate supersaturation, which at the values indicated may well have stressed the fish. Precautions were however taken to avoid supersaturation, including vigorous aeration of sump water, sealed pumps to avoid cavitation and an open head

tank to allow pumped water to come into contact with air before entering the treatment tanks. An inaccurate DO meter, as indicated by dissimilar replicate results and high DO values in water left to stand, was the likely cause of unrepresentatively high DO levels.

Pronounced diurnal fluctuations in DO saturation levels were shown to occur, similar to those described by Honer et al. (1987a), though cyclical changes were not proven. DO in the tanks and effluent fell soon after feeding, probably due to an increase in fish metabolic rate (Rosenthal et al., 1981), increased biofilter bacterial activity and bacterial population size. Critical DO levels did not occur, probably because stocking densities in each tank were low. After 4.5 h following feeding, DO saturations rose to an overnight level, probably due to slowing of fish metabolic rate, decreased filter activity and the action of water aeration systems. DO levels appeared to decrease at about 04.00 h, coincident with increased water temperature. The increase in temperature was small and would not have increased percentage saturation greatly. It is possible that an increase in fish activity at first light may have caused the reduction, but there are no behavioural observations to corroborate this theory.

A clear association between water depth and DO was evident. Both the shallowest tanks (3 cm depth) contained water with the lowest DO saturation compared with other

tanks of the same residence time or flow rate. Tanks 7 and 3, which had similar flow rates (2.00 and 1.71 l min^{-1} respectively) but different residence times, depths (3 and 9 cm respectively) and therefore volumes, contained water with a similar DO concentration. So in this case DO was not related to residence time, depth and volume. Tanks 1 and 9, with similar residence times (10.84 and 9.27 min respectively) but different flow rates and depths (3 and 9 cm respectively), did not however contain water with a similar DO concentration: tank 1 with the lower flow rate had a lower DO concentration. Flow rate was therefore probably a more important determinant of DO than residence time, depth, or volume.

An increase in flow rate would increase the quantity of incoming water of a higher DO concentration. At greater stocking densities DO will be used up quicker, so flow rate will have to be increased. It should be noted that only at the most shallow depth was DO greatly reduced. At greater depths, even within the small range investigated in this study, little difference in DO was achieved. An alternative conclusion may then be drawn. Assuming that sufficient depth is provided, to act as a reservoir of oxygen for the stocking density of fish present, flow rate may be reduced. Careful adjustment of flows to ensure that sufficient DO is provided to the fish will be required.

An obvious method of increasing DO in a tank is to

provide aeration directly into the tank. It was expected that this would however produce highly variable and complex mixing effects which could not be quantified in this study, so no aeration was added directly to the treatment tanks.

8.5.12 Total and unionized ammonia

Colt and Armstrong (1981) suggested that NH_3 toxicity was the second most limiting factor in intensive fish culture after DO. NH_3 has a higher cell membrane permeability and lipid stability compared with NH_4^+, as summarised by Poxton and Allouse (1982). NH_4^+ was believed by Colt and Armstrong (1981) to have an important effect on fish culture, though Sheehan and Lewis (1986) showed that concentrations of up to 1787 mg NH_4^+-N l^{-1} were not toxic to channel catfish at 21 ^oC. NH_3 is therefore considered to be the more toxic of the two forms.

Lethal or sub-lethal levels of NH_3 are difficult to define accurately because the toxic effects of this substance are dependant on pH and CO_2 (Alabaster and Herbert, 1954), oxygen concentration (Downing and Merkens, 1955), exposure period (Burrows, 1964) and acclimation (Lloyd and Orr, 1969). Meade (1985) suggested that a maximum safe concentration was unknown because chronic gill hyperplasia was not caused by NH_3 alone, as was previously thought. Wickins (1981) and Poxton and Allouse

(1982) reviewed the effects of NH_3-N concentrations in marine and freshwater fish.

Alderson (1979) found that during an 11 day period 0.08 and 0.14 mg l^{-1} NH_3-N produced no effect on growth of turbot at pH 6.8 and 7.9 respectively, while 0.30 and 0.90 mg l^{-1} NH_3-N resulted in no growth. Poxton and Allouse (1982) suggested tentative maximum allowable NH_3-N levels of 0.025 mg l^{-1} NH_3-N at pH 6.8 and 0.075 mg l^{-1} NH_3-N at pH 7.9, derived by applying the safety factor suggested by EIFAC (1970) to the data of Alderson (1979). Wickins (1981) suggested a more generally applicable maximum tolerable NH_3-N concentration of 0.1 mg l^{-1} NH_3-N, but did not indicate an exposure period length.

The maximum level of NH_3-N which was detected was 0.006 mg l^{-1}. This concentration was less than the limits recommended above, so NH_3 was considered unlikely to have stressed the turbot during this experiment. It should be noted that even in the shallowest of tanks (3 cm) with the slowest flow rate (0.57 l min^{-1}), NH_3 concentrations were not likely to have been stressful. A differential effect of depth on NH_3 concentration leading to differential secondary effects on various biological parameters, were also unlikely. A study of NH_3 fluctuations in this experiment may however indicate expected fluctuations, due to similar treatments, in other situations where NH_3 concentration is critical.

Fluctuations in total ammonia (NH_4), which includes both NH_3 and NH_4^+, are a useful indication of the level of nitrogenous metabolites present in the system and are comparable with other studies in which NH_4 rather than NH_3 was described. NH_4 fluctuations during the day were shown to be influenced by the time of feeding. General periods of relatively low and stable NH_4 concentrations overnight and prior to feeding time were followed by a period of relatively high and greatly fluctuating NH_4 concentrations, peaking up to 4.5 h after feeding. Similar fluctuations have been described by: Rosenthal et al. (1981) studying an intensively cultured recycle system; Honer et al. (1987a) who reared juvenile tilapia Sarotherodon galilaeus and Poxton and Allouse (1987) studying fluctuations in ammonia and nitrite in a recirculation system for culturing turbot. Clearly then the concentration of NH_4-N is related to the digestive activity of the turbot in breaking down proteins and other nitrogenous compounds in the feed. The lag between feeding and the peak in NH_4-N was related to the speed of the digestion of the fish. After excretion, NH_4-N is nitrified by the increasing population of bacteria in the biofilters, so water flowing into the tanks will have a lower NH_4-N concentration than the tank water, which it will dilute. This is combined with a decrease in NH_4-N production as the last of the food is digested, to produce a decrease in the NH_4-N concentration. Honer et al. (1987a) found a logarithmic decrease, which they also attributed to a reduction in digestive activity and

dilution with influent water. During this study, the influent water NH_4-N concentration was not noticeably lower than the concentration in the water inside the tanks after feeding. The decline in NH_4-N concentration within the tanks, as opposed to the system as a whole, after the maximal values may therefore be primarily due to a decrease in the digestive activity of the fish.

The maximum NH_4-N concentration achieved and length of time after feeding that the maximum concentration occurred, appeared related to the flow rate, depth or volume of the tank, in tanks with the same residence time. The maximum concentration was highest and occurred later after feeding in the shallowest tank with the lowest flow rate. Poxton and Allouse (1987) attributed the amplitude and timing of the NH_4-N fluctuations to flow rate, temperature, mean weight and biomass of fish and the efficiency of the biofilters. In the present study all these parameters can be considered constant except for flow rate, and so with this one exception are unlikely to have caused any differences in NH_4-N concentrations. Honer et al. (1987a) found that an increase in flow rate reduced the peak in the NH_4-N cycle, confirming results found in the present study. In tanks with the same flow rate, no influence of either residence time, depth or volume was observed. It seems likely then that flow rate was one of the main factors responsible for determining the amplitude and timing of the NH_4-N peak after feeding, indicating that influent dilution rather than digestion

rate was the dominant factor in determining NH_4-N concentration within the tanks during this experiment. This does not preclude the influence of other factors, listed previously, such as biomass, in other situations in which they have not been maintained constant.

Assuming the results of the 24 h water quality survey were representative of other days during the experiment, two consequences of this diurnal fluctuation in NH_3 and NH_4^+ concentration are important. Firstly, as suggested by Poxton and Allouse (1987), random water quality sampling during the day may produce misleading results. Secondly, a knowledge of the pattern of NH_3 or NH_4 fluctuations during a day, or other period, allows the prediction of peak values from one measurement, as suggested by Honer et al. (1987).

8.5.13 Nitrite

Nitrite occurred in the culture system as a result of the nitrification of ammonia by bacterial populations in the biofilter. The quantity of nitrite present in a recycle system is related to the biomass of fish, species and biomass of filter bacteria, and therefore is also related to the design of biofilter and the pattern of fluctuations of NH_4-N concentration which influences bacterial populations.

Nitrite toxicity has been shown to be related to

species (Brownell, 1980a), chloride concentration (Perrone and Meade, 1977), calcium (Crawford and Allen, 1977) and pH (Colt and Tchobanoglous, 1976) at values outside of the range 7.5 - 8.5 suggested by Russo and Thurston (1977). Wickins (1981) suggested that a level of 1.0 mg l^{-1} NO_2-N should not be exceeded in seawater, but Eddy et al. (1983) regarded NO_2 as being harmless in all waters except those with a very low Cl^- concentrations, ie. not seawater. This suggestion is supported by results of Brownell (1980a) who stated 24 h LC_{50} NO_2-N concentrations of 1230 mg NO_2-N l^{-1} for marine fish larvae.

Of the NO_2-N concentrations determined during the experiment, none were found to exceed one tenth the most conservative maximum recommended level proposed by Wickins (1981). NO_2-N concentration was therefore unlikely to have stressed the turbot and was not expected to have influenced the biological parameters measured.

A clear pattern of diurnal fluctuations related to feeding or treatment was not observed, mainly due to the apparently large fluctuations in NO_2-N concentration with time. Examination of the magnitude of the fluctuations indicated that values varied within 0.035 mg NO_2-N l^{-1}, which was a small range compared with the maximum recommended level. It may be that NO_2-N concentrations varied little throughout the experiment, so significant differences between tanks were either too small to be defined or did not occur.

A peak in NO_2 concentration which occurred in four of the six tanks was observed 11.5 h after feeding and approximately 7 h after maximum NH_4-N levels. The 7 h lag between NH_4-N and NO_2 maxima was probably the result of the time taken for the nitrifying bacteria populations to build up and convert NH_4-N into NO_2.

8.5.14 Water quality parameters conclusions

1. Temperatures within 3 ^{o}C of the optimum of 18 ^{o}C did not stress the fish.
2. pH varied between 7.86 - 8.16, within the recommended range of 6.5 - 8.5 and so did not stress the fish.
3. pH decreased slowly throughout the experiment and daily decreases were related to feeding.
4. pH was lowest and decreased most rapidly in tanks with the shallowest water depth (3 cm).
5. An adequate water volume was required to maintain pH levels, but pH reduction may be overcome by increasing flow rate.
6. Salinity at 35 ppt +/- 0.8 ppt did not stress the fish.
7. DO did not fall below 90 % saturation, 8 % greater than the minimum recommended value at a similar temperature.
8. Some supersaturation of water may have occurred, which would have stressed the fish, though this occurrence was considered unlikely.

9. DO saturation in the tanks and effluent fell 1.5-2.5 h after feeding and rose again approximately 4.5 h after feeding.
10. The two shallowest tanks (3 cm) contained water with the lowest DO, compared with other tanks of the same residence time or flow rate.
11. Increased flow rate at a particular residence time, with an associated increase in depth increased DO concentration.
12. Flow rate was probably a more important determinant of DO than residence time, depth or volume. An increase in flow rate increased DO.
13. Maximum NH_3-N concentrations were less than one fortieth of the maximum recommended concentration of 0.025 mg NH_3-N l^{-1}, and so did not stress the turbot.
14. Low and stable NH_4-N and NH_3-N concentrations occurred overnight and prior to feeding time, followed by a period of higher and greatly fluctuating concentrations, peaking up to 4.5 h after feeding.
15. Flow rate was probably mainly responsible for determining the amplitude and timing of the NH_4-N and NH_3-N peaks after feeding: the maximum concentrations were highest and occurred later in the shallowest tank with the lowest flow rate.
16. Maximum NO_2-N concentrations were less than one tenth the maximum recommended level of 1.0 mg NO_2-N l^{-1}, and so did not stress the turbot.

17. Maximum NO_2-N concentrations probably occurred 11.5 h after feeding and approximately 7 h after maximum NH_4-N levels.
18. Critical levels of water quality during the experiment were not detected. pH, DO, NH_4-N and NH_3-N conditions were marginally better in the deeper tanks and in tanks with a faster flow rate. However in all but the shallowest, 3cm deep tanks, water quality was statistically similar.

8.5.15 Behaviour

A strictly controlled experiment to determine the behavioural responses of the juvenile turbot to different depths of water was not conducted, but casual observations were made. The main effect of depth on behaviour appeared to be associated with feeding. The moist pellets used during the experiment were negatively buoyant and sank immediately on hitting the water. The speed of the sinking pellets could be minimized by releasing the pellet from as low as possible above the top of the water. Turbot, as visual feeders, have a feeding response to a moving target. In the shallower tanks the fish have less time to strike the pellet as it falls. In all but the 3 cm deep tanks, few pellets which lay on the bottom were eaten, unless fish movement caused them to be resuspended. In the 3 cm deep tanks there was a clear tendency to eat pellets on the bottom. This unusual response may have been forced upon the fish by hunger resulting from

obtaining few falling pellets. The characteristic movement of the fish from the substrate to mid- and surface water at feeding time was evident in all tanks.

These observations and the lack of a significant association between food consumption and water depth indicate that the turbot were capable of feeding, even in tanks where the water level only just covered the fish.

8.5.16 Hydrodynamics conclusions

Chapter 5 describes experiments designed to define the mixing characteristics of tanks managed in the same way as during the experiment described in the present chapter, ie. the same range of depths, flow rates and residence times. Section 5.7 summarises the conclusions from this hydrodynamics work, which to aid comparison with biological and water quality data will be summarised here.

1. In tanks with the same flow rate, mixing improved significantly with a decreased depth of water within the range 9 - 18 cm and an associated decrease in residence time.
2. A depth of water between 2.89 cm and approximately 9 cm was required to minimise or eradicate dead volume in the treatment tanks at 2 l min^{-1} flow rate. At greater depths, dead volumes would be expected and at shallower depths no further decrease in dead volume would be achieved.

3. Mixing was not influenced by depth changes, within the range 6 - 18 cm, with constant residence time and variable flow rates.
4. Adjustment of residence time probably influenced mixing more than flow rate adjustments, within the depth range 9 - 18 cm.

8.5.17 Combined discussion of biological, water quality and hydrodynamic parameters

Mixing generally increased with decreased depth and decreased residence time at a constant flow rate. However improved conditions of pH, DO, NH_4-N and NH_3-N were obtained by increasing depth. It seems likely then that these improvements in water quality may not have resulted from increased mixing within the tank as would be expected. It is possible that the effect of dilution of tank water, high in nitrogenous metabolites, with a low pH buffering capacity and low in DO, may be a more important aspect than tank mixing efficiency. This is confirmed by the suggestion that flow rate was probably the dominant factor influencing DO, NH_4-N and NH_3-N. It may be that dead volumes in the deeper tanks act as a reservoir of DO and pH buffers, and contain water with low NH_4-N and NH_3-N levels, when stocking densities are low, rather than containing water low in DO, low in pH and high in NH_4-N and NH_3-N. The greater volume of the deeper tanks act as a buffer or damper against large fluctuations in water quality, spreading influent variations over a longer

period. Stocking density was not high in this experiment so it is possible that dead volumes of water, which in a higher stocking density situation, would have contained poor quality water, may contain good quality water and hence act as the reservoir suggested.

There was a tendency towards higher growth rates in the deeper tanks, in which water quality was marginally better yet fluid mixing was worse, so it appears that it is water quality, in particular pH, DO, NH_4-N and NH_3-N which may have influenced fish growth rather than the tank hydrodynamics. This would be expected as tank mixing would have to be extreme to directly influence the biological parameters. Water quality was probably a secondary effect of the tank hydrodynamics, though in the range of treatments used during this experiment, hydrodynamics had little effect on water quality except at the lowest flow rates.

Definite links between water quality and biological parameters during this experiment are difficult to draw because it has been shown that water quality was unlikely to have stressed the fish and because few incidences of stress coincident with poor water quality were found. It is expected that if the hydrodynamics or stocking density had been more extreme, the water quality would have reached stressful levels in some of the tanks, resulting in differential biological parameters such as growth.

A more positive conclusion is that despite a depth range of 3 - 18 cm under the conditions stated, the growth and condition of the fish were not often affected greatly, except at the shallowest depth. In certain conditions it may then be feasible to reduce water depth, so that either flow rate can be reduced, so conserving water, whilst maintaining the same residence time, or flow rate can be maintained so decreasing the residence time, increasing flushing. Results of this experiment suggest that the latter option may be most beneficial as it was probably flow rate rather than hydrodynamic mixing which was important.

Care should however be taken when applying conclusions drawn from this study with other situations. In particular, a reduction in depth in tanks with a different shape, or managed differently, to those described above may have a different effect on water mixing. Different stocking densities would be expected to have different influences both on tank mixing and water quality.

8.6 CONCLUSIONS

In general: the hydrodynamics of the treatment tanks improved with decreased depth; water quality declined at the shallower depths; fish growth and FCR was occasionally better in the tanks filled to a greater depth. Flow rate rather than hydrodynamic mixing appeared a more important

influence on water quality, which in turn may have influenced fish growth. Water quality however rarely reached critical levels during this study and so little differential effect on turbot growth and fitness was found. It is therefore proposed that during this experiment the range of treatments adopted were insufficient to cause pronounced critical levels of water quality and as a result little influence of depth on various indicators of fish growth and fitness occurred.

CHAPTER 9

WATER DEPTH EXPERIMENT 2

CONSTANT STOCKING DENSITY BY VOLUME

9.1 INTRODUCTION

To determine the influence of water depth, at a constant SDv, on biological, water quality and hydrodynamic parameters, and to establish if the cause of the poorer water quality in the shallower tanks, described in Chapter 8, was due to increased SDv, duplicate experiments to those in Chapter 8 were conducted, but with SDv rather than SDa maintained the same in all treatments.

9.2 OBJECTIVES

The aims of the investigations reported in this chapter were:

1. To quantify the influence of water depth, in a defined culture tank, on the biological aspects of turbot culture, ie. growth rate, conversion efficiency, condition factor and behaviour, at a constant SDv.
2. To quantify the influence of water depth, in a defined culture tank, on water quality, ie. temperature, salinity, pH, DO, NH_4, NH_3 and NO_2 at a constant SDv.
3. To determine the inter-relationships between the biological, water quality and hydrodynamic parameters

at different depths of water, at a constant SDv.

4. To establish if the cause of the poorer water quality in the shallower tanks described in Chapter 8, was due to increased SDv.
5. To partially replicate experiments described in Chapter 8.

9.3 MATERIALS AND METHODS

9.3.1 Summary of method

Experiments were set up in which turbot were grown in a range of water depths in the treatment tanks used throughout this study. All tanks contained turbot at the same stocking density, calculated as a factor of the tank working volume of water. Five of the tanks, with a range of water depths, were maintained at the same flow rate and five tanks, with a replicate range of water depths were maintained at the same residence time. A comparison of data from different treatments was made, to investigate the influence of depth on various biological, water quality and hydrodynamic parameters whilst allowing for other, possibly conflicting variables, ie. stocking density, flow rate and residence time, which could not be maintained the same in all the treatments.

9.3.2 Treatments

Experiments were conducted during a 57 day period

from 4/2/88 (day 0) to 31/3/88 (day 56).

The recirculation system described in Chapter 4 (Figure 4.1) was used. Five treatment tanks, described in Chapter 4, were filled with seawater to depths of either 3, 6, 9, 12, or 18 cm and flow rates were a constant 2 l min^{-1} into each tank. Five other tanks were maintained at replicate depths, but with a constant residence time (ideal mean residence time, t_i) of 10.8 min. To aid comparisons between the results of WDE1 and WDE2, two treatments were maintained as similar as possible in both studies. Tanks 3 and 9 contained 10 fish in both studies, though the mean weight of the fish was necessarily different. Flow rates, residence times and SDs are summarized in Table 9.1.

Table 9.1: Treatment tank experimental regime for WDE2

Tank no.	Depth (cm)	Flow (l min^{-1})	Residence time (min)	Fish (no.)
1	3	0.57	10.84	3
2	6	1.14	10.84	7
3	9	1.71	10.84	10
4	12	2.28	10.84	13
6	18	3.42	10.84	20
7	3	2.00	3.09	3
8	6	2.00	6.18	7
9	9	2.00	9.27	10
10	12	2.00	12.36	13
12	18	2.00	18.54	20

Tank volume = 2.06 l cm $depth^{-1}$
SDv = 1.11 fish cm $depth^{-1}$

9.3.3 Fish

Post-weaned turbot within the size range of 4.1-30.9 g, at the start of the experiment, were supplied by Golden Sea Produce of Hunterston, Scotland (see Chapter 4). SDv of all tanks in this study was calculated, as follows, to be the same as the 9 cm deep tanks which contained 10 fish. Tank volume increased as a factor of water depth so using water depth for the calculation:

$$\text{In a 9 cm deep tank SDv} = \frac{10 \text{ fish}}{9 \text{ cm}} = 1.11 \text{ fish cm depth}^{-1}$$

Therefore the number of fish in each tank = depth x 1.1

To maintain a similar SDv in all tanks, turbot were stocked at the nearest whole fish to 1.1 fish cm $depth^{-1}$. Fish were assigned to each tank randomly using random number tables (Murdoch and Barnes, 1970), so an approximately similar initial SDv (g cm^{-3}) was assumed to have occurred in all tanks. Equal numbers, to the nearest whole fish, of malpigmented and pigmented fish were assigned to each tank, so a stratified random assignment method was employed.

The fish were acclimated to the recirculation system for a period of 2 weeks. During acclimation the fish were held in two treatment tanks maintained at a depth of 16 cm and a flow rate of approximately 4 l min^{-1}. Fish were not acclimated to the particular treatments of the experiment

because it was considered that this would reduce possible stress differences due to treatment.

9.3.4 Feed

The feeding regime and food composition was the same as that described in section 8.3.4 and Tables 4.2 and 4.3, except that feed moisture content was 41.3 %.

9.3.5 Measurements and calculations

It was planned that at the beginning of, and every 2 weeks throughout the experimental period of 8 weeks, fish weight and length measurements would be taken. Due to illness of the author however, the third set of measurements was conducted on Day 33, 5 days late. Subsequent growth periods were reduced in length accordingly, to ensure the correct number of measurements were taken within the overall experimental period. All experimental fish were treated the same, so no differences in results occurred between different treatments, due to measurement period variation. The number of fish measurements within the 56 day period was the same as in WDE1 and growth periods approximated 14 days.

The wet weight and total length of each fish was measured, using a method designed to reduce stress (see section 4.5.1) used in WDE1. The tank number and

pigmentation type corresponding to these parameters was noted.

Biomass, biomass change since previous measurement, growth rates, condition factors, and conversion efficiencies were calculated, as described in Chapter 4. The number of fish in each treatment tank and the length of the growth periods were variable, so biological data was expressed as a group mean, or per unit time respectively, eg. fish weight gain under different treatments or during different growth periods was not comparable, but mean weight gain per treatment or per day was comparable both in time and treatment.

For simplicity and to ensure comparability, the general scheme of data analysis described in section 8.3.5 was employed with the above minor variations to the method.

9.3.6 Water quality

To assess differential water quality levels and changes in the experimental system, three series of water quality samples were collected and analysed as described in section 4.6. Three of the series of samples described in WDE1 (section 8.3.6) were replicated during this study: samples taken daily at 11.30 h; 24 h samples, taken during days 52 - 53; and within tank samples, taken during day 43. The 4 h sample series described in section 8.3.6 was

not replicated in this study because previous results were shown to be of limited value.

9.4 RESULTS

Using a similar method to that described in section 8.4, due to the large number of parameters studied and the complexity of the possible inter-relationships between these parameters, statistical analyses were concentrated on the biological parameters of fish weight, fish length, condition factor and specific growth rate unless the data suggested that further investigations were required.

9.4.1 Survival

No fish died throughout the experimental period.

9.4.2 Fish weight

Histograms (Figures 9.1 - 9.5) and normal probability plots were drawn of the individual weights of all experimental fish at each measurement period. Both types of plot indicated that the distribution of weights could be considered normal throughout the experiment and therefore normal statistics were applicable. A positive skew in the distribution, evident at the start of the experiment, decreased with time, indicating a gradual reduction in the proportion of the total sample which was heavier than would be expected in a perfectly normal

Figure 9.1. Histogram of fish weights - day 0

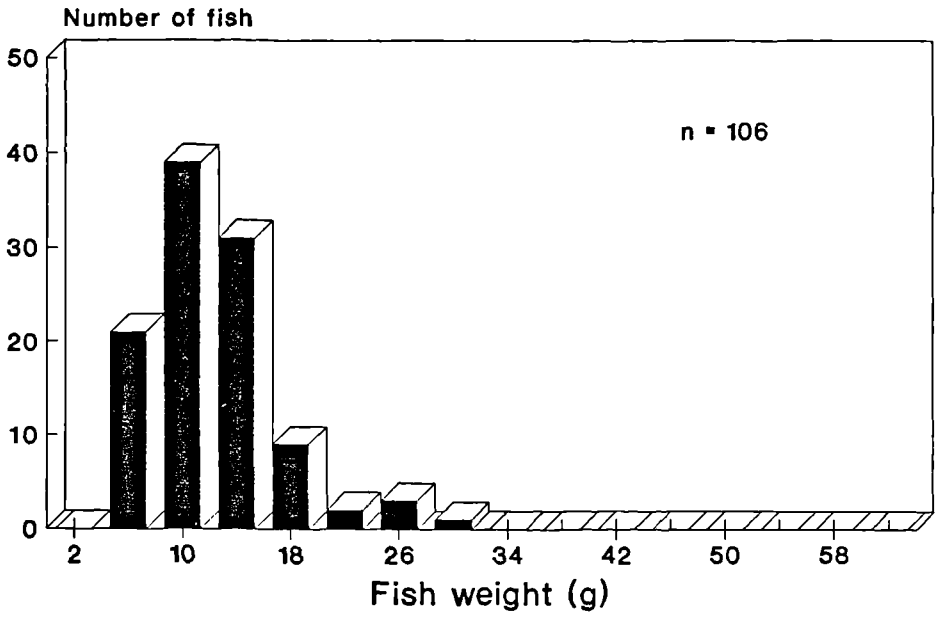


Figure 9.2: Histogram of fish weights - day 14

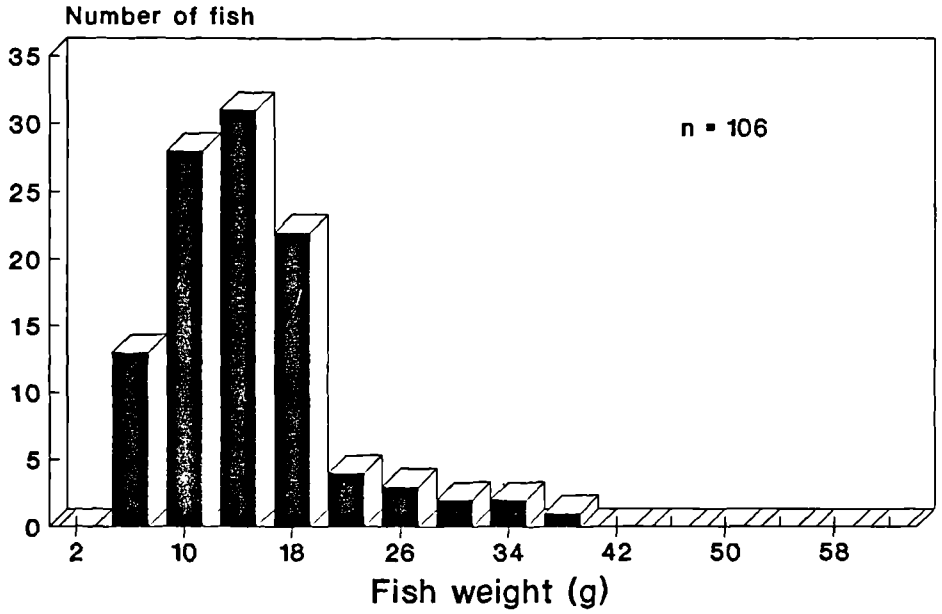


Figure 9.3: Histogram of fish weights - day 33

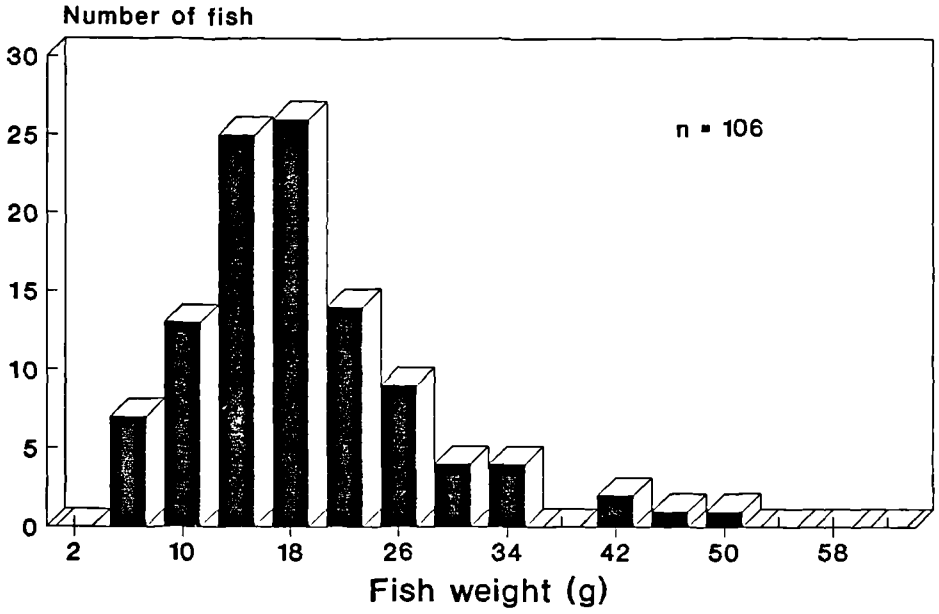
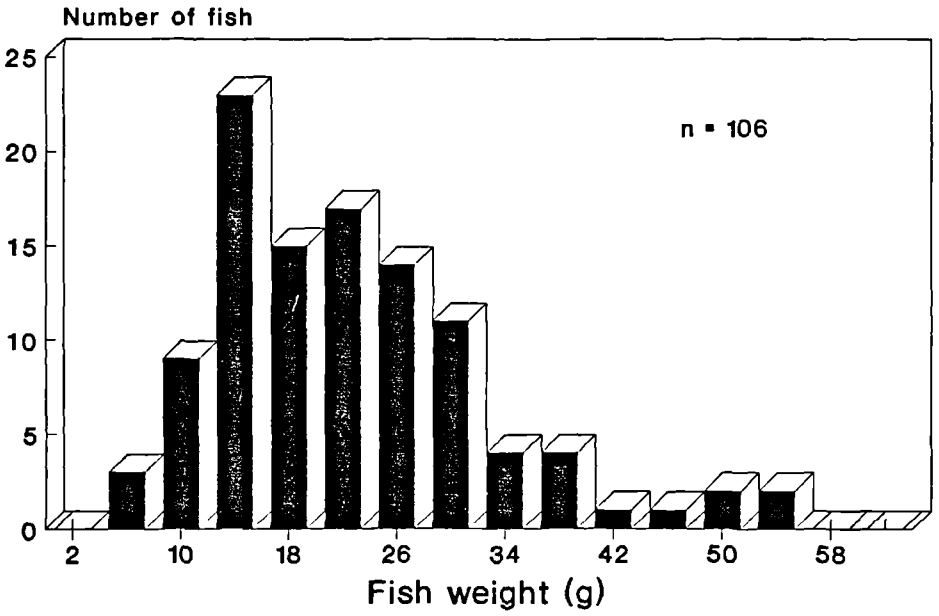


Figure 9.4: Histogram of fish weights - day 46



population. Individual fish weight varied between 4.1-30.9 g at day 0, to 6.2 - 62.4 g at day 56.

Two-sample t-tests were calculated, comparing the mean weights of pigmented and malpigmented fish in each treatment tank at each measurement day. The null hypothesis of the tests was that there was no difference in the mean weights of the two groups of fish. Results indicated that the mean weights of the two pigmentation types of fish were not significantly different throughout the experiment. The mean weight of fish in each tank was not therefore influenced by fish pigmentation, so the weights of pigmented and malpigmented fish from the same tank were combined for further analyses.

The influence of water depth on fish weight was investigated using plots, correlation and regression analyses, which quantified the distribution of tank mean fish weights with tank water depth during the same day and within each flow type during the same day. Results indicated that tank mean fish weight was not associated with water depth at any time during the experiment. Separation of results into either those which originated from tanks with the same flow rate or the same residence time, also produced no significant associations between tank mean fish weight and water depth.

Figure 9.6 illustrating the distribution of tank mean fish weights in tanks containing different depths of

Figure 9.5: Histogram of fish weights - day 56

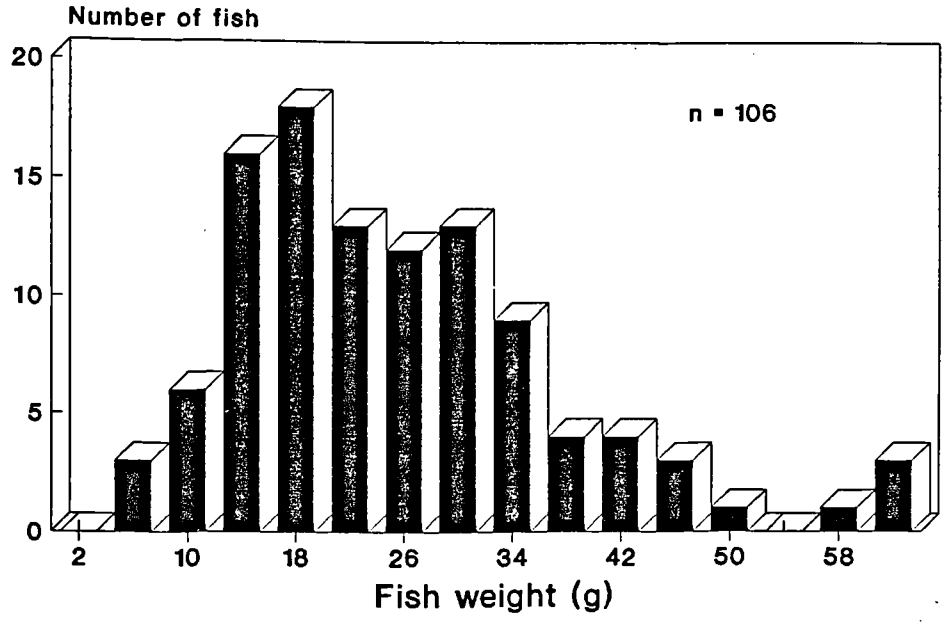
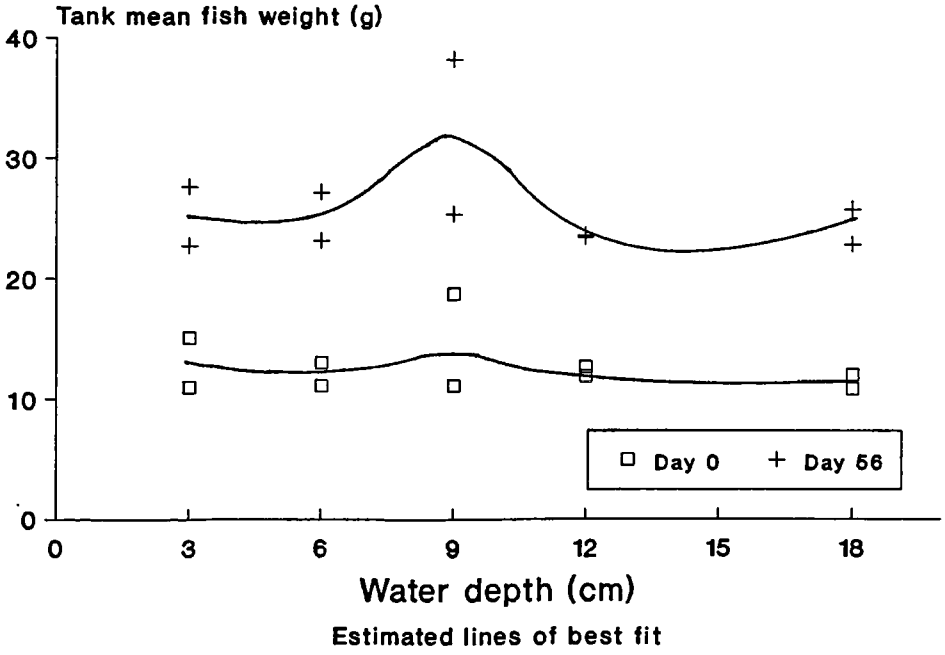


Figure 9.6: Tank mean fish weight distribution at different water depths

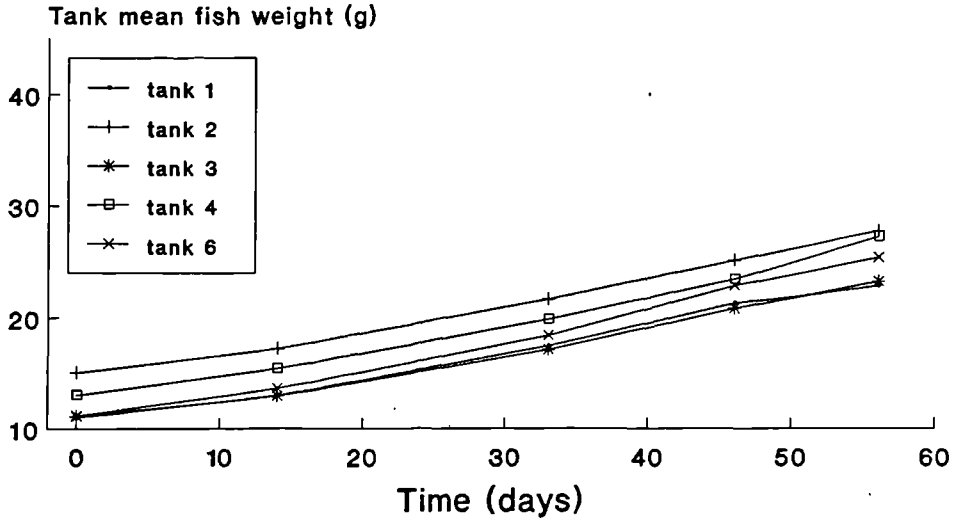


water, indicates that the fish populations in the tanks were established at day 0 with a marginally decreasing mean weight with increased water depth. This trend became less prominent with time and by the end of the experiment the range of mean weights, with the exception of tank 6, was only 5.03 g.

Results of correlation and regression analyses summarized in Table 9.2 indicate that, in all tanks, the individual fish weights increased significantly throughout the experiment. The regression equations corresponding to the results shown in Table 9.2 are listed in Table 9.3. Comparisons between the rate of increase in weights of individual fish in different tanks, as quantified by the gradient of the regression lines, indicated that fish in tank 7 (3 cm depth) increased in weight significantly faster than fish in tanks 3, 4, 6, 8, 9, 10 and 12. Fish in tank 4 (12 cm depth) increased in weight significantly faster than fish in tank 8 (6 cm depth).

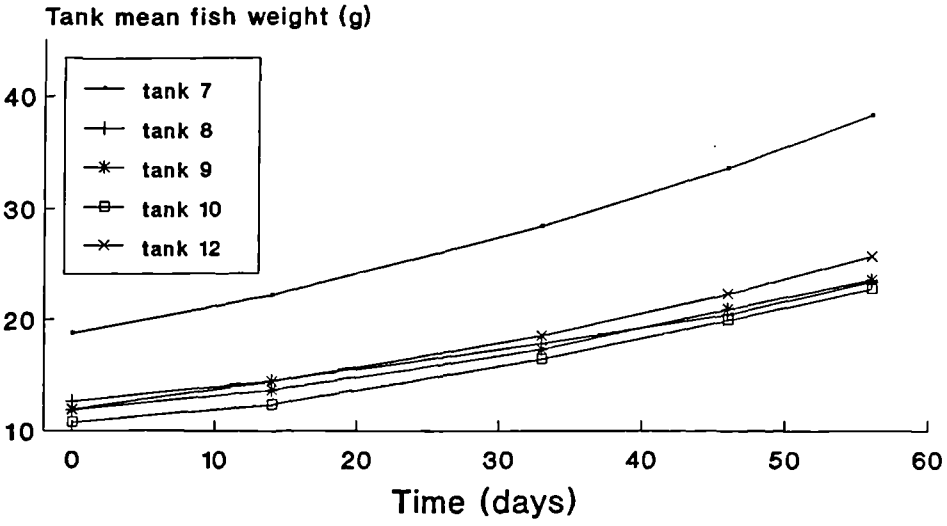
Figures 9.7 and 9.8 illustrate the tank mean fish weight fluctuations with time, showing an increase in overall mean fish weight from 12.7 g at day 0 to 26.0 g at day 56.

Figure 9.7: Tank mean fish weight increases with time - same residence time tanks



Water depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 6 - 18cm

Figure 9.8: Tank mean fish weight increases with time - same flow rate tanks



Depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 12 - 18cm

Table 9.2: Changes in individual fish weights with time at different water depths

tank no.	water depth (cm)	d.f.	r	regression p
1	3	13	0.537	**0.039**
2	6	33	0.362	**0.033**
3	9	48	0.529	**<0.001**
4	12	63	0.668	**<0.001**
6	18	98	0.510	**<0.001**
7	3	13	0.543	**0.036**
8	6	33	0.375	**0.027**
9	9	48	0.474	**0.001**
10	12	63	0.663	**<0.001**
12	18	98	0.460	**<0.001**

Table 9.3: Regression equations describing the significant increase in individual fish weights (y) with time (x)

tank no.	water depth (cm)	regression equation
1	3	y = 10.5 + 0.222 x
2	6	y = 14.5 + 0.229 x
3	9	y = 10.4 + 0.221 x
4	12	y = 12.3 + 0.250 x
6	18	y = 10.5 + 0.260 x
7	3	y = 17.8 + 0.347 x
8	6	y = 12.1 + 0.188 x
9	9	y = 11.1 + 0.211 x
10	12	y = 9.97 + 0.216 x
12	18	y = 11.3 + 0.243 x

9.4.3 Fish length

Histograms and normal probability plots were drawn of the individual fish lengths at each measurement period.

Both types of plot indicated that the distribution of lengths could be considered normal throughout the experiment and therefore normal statistics were applicable. No skew in the distribution of lengths was evident. The range of fish lengths during the experiment was between 60 - 113 mm at day 0, to 71 - 148 mm at day 56.

Two-sample t-tests were calculated, comparing the mean lengths of pigmented and malpigmented fish in each treatment tank at each measurement day. The null hypothesis of the tests was that there was no difference in the mean lengths of the two pigmentation groups of fish. Results indicated that the mean lengths of the two pigmentation type groups of fish were not significantly different at any time during the experiment. Tank mean fish length was not therefore influenced by pigmentation, so the lengths of pigmented and malpigmented fish from the same tank, were combined for further analyses.

The influence of water depth on fish length was investigated using plots, correlation and regression analyses which quantified the distribution of tank mean fish lengths with respect to tank water depth during the same day and within each flow type group of tanks during the same day. Results indicated that tank mean fish length was not associated with water depth. Separation of results into either those from the same flow rate or the same residence time tanks, also produced no significant

associations between tank mean fish length and water depth.

Tank mean fish length distribution with respect to water depth (Figure 9.9), followed a similar pattern to that of mean fish weights, indicating that fish populations in the tanks were set up at day 0 with a marginally decreasing mean length with increased depth. This trend altered little throughout the experiment.

Results of correlation and regression analyses summarized in Table 9.4 indicate that, in all tanks, fish length increased significantly throughout the experiment. The regression equations corresponding to the results shown in Table 9.4 are listed in Table 9.5. Comparisons between the rate of increase in the lengths of individual fish in different tanks, as quantified by the gradients of the regression equations (b), indicate that the increase in individual fish length was statistically similar in all tanks.

Figures 9.10 and 9.11 illustrate the tank mean fish length increases with time, showing an increase in overall mean fish length from 87 mm (+/- sd 10.3) at day 0 to 109 mm (+/- sd 16.3) at day 56.

Figure 9.9: Tank mean fish total length distribution at different water depths

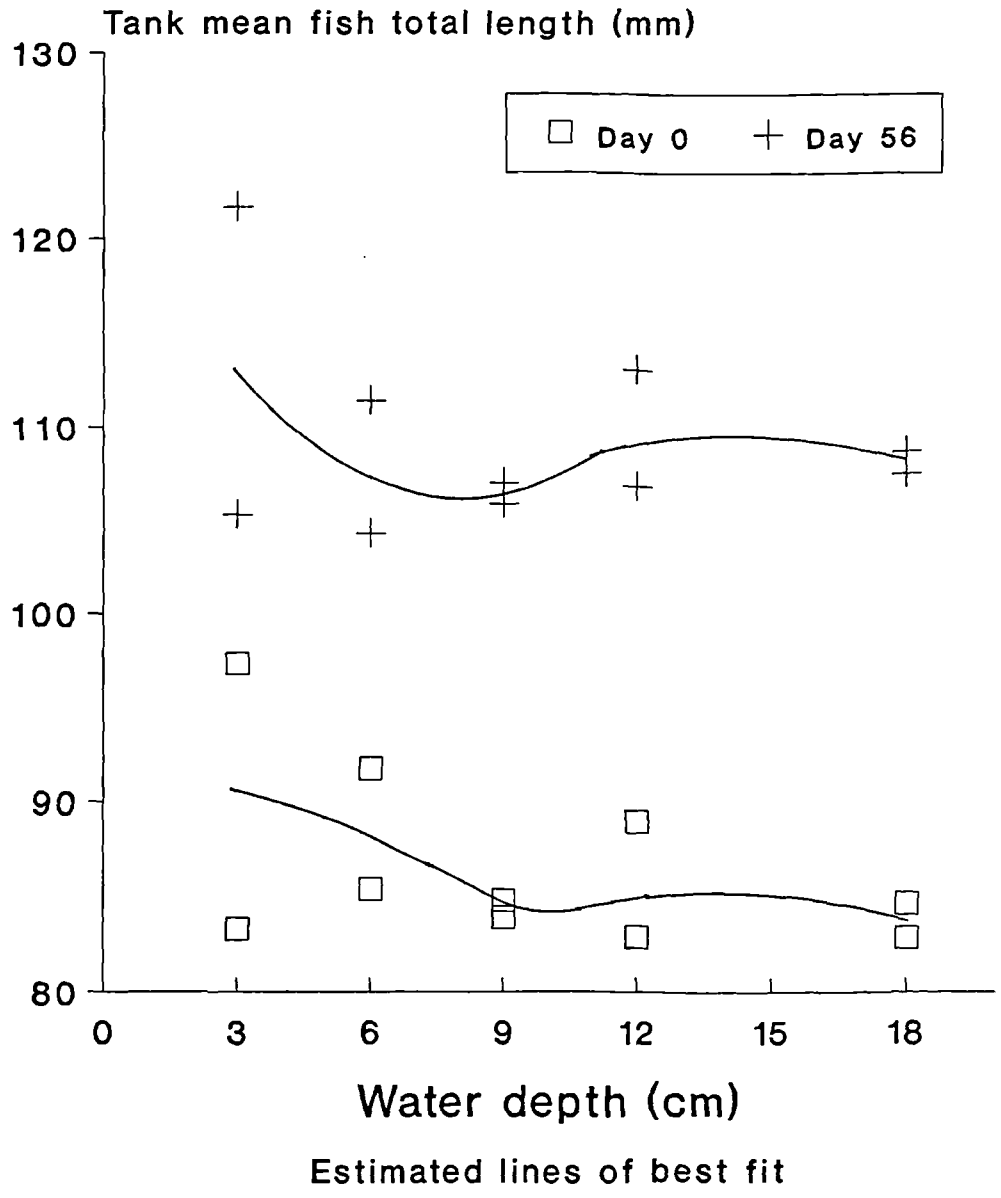
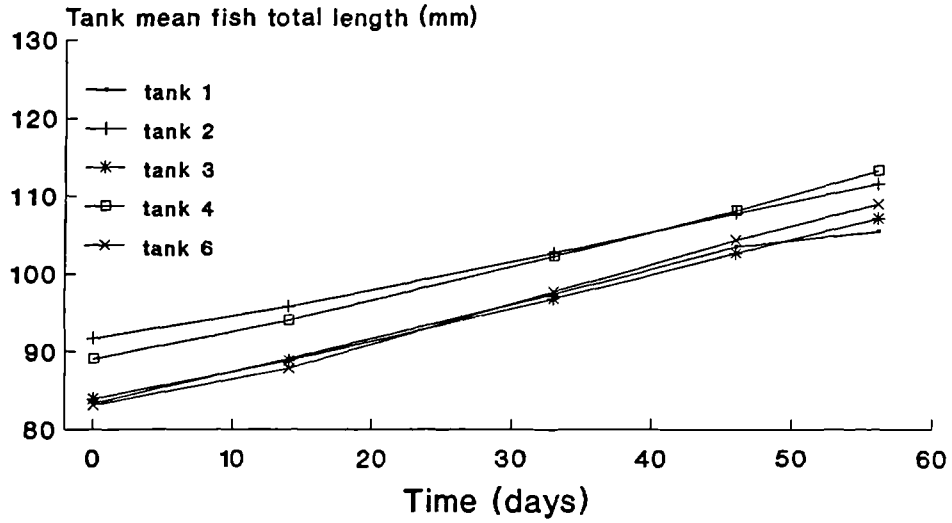
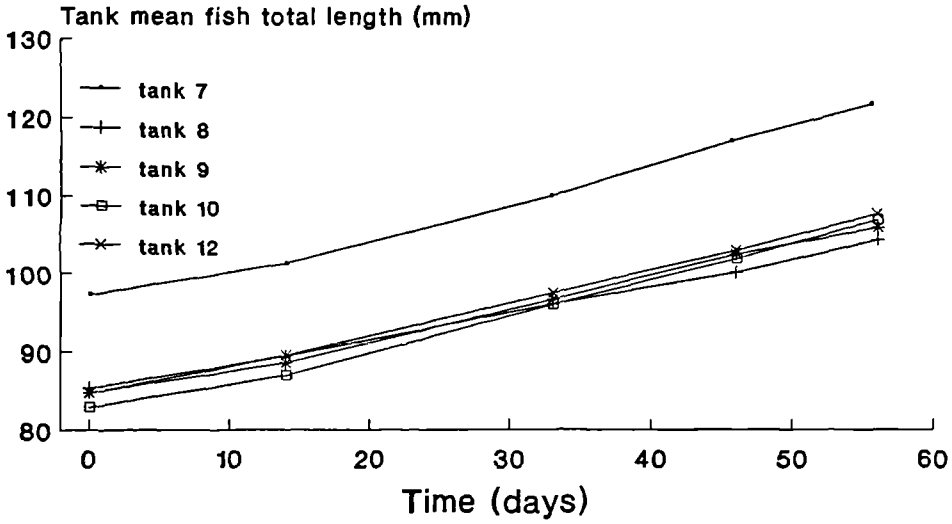


Figure 9.10: Tank mean fish total length increases with time - same residence time tanks



Water depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 6 - 18cm

Figure 9.11: Tank mean fish total length increases with time - same flow rate tanks



Water depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 12 - 18cm

Table 9.4: Changes in individual fish lengths with time

tank no.	water depth (cm)	d.f.	r	regression p
1	3	13	0.553	**0.033**
2	6	33	0.420	**0.012**
3	9	48	0.608	**<0.001**
4	12	63	0.694	**<0.001**
6	18	98	0.548	**<0.001**
7	3	13	0.665	**0.007**
8	6	33	0.401	**0.017**
9	9	48	0.494	**<0.001**
10	12	63	0.711	**<0.001**
12	18	98	0.494	**<0.001**

Table 9.5: Regression equations describing the significant increase in individual fish lengths with time

tank no.	water depth (cm)	regression equation
1	3	y = 83.5 + 0.408 x
2	6	y = 91.2 + 0.355 x
3	9	y = 83.4 + 0.415 x
4	12	y = 88.4 + 0.430 x
6	18	y = 82.2 + 0.473 x
7	3	y = 96.2 + 0.445 x
8	6	y = 85.1 + 0.401 x
9	9	y = 84.1 + 0.389 x
10	12	y = 82.0 + 0.434 x
12	18	y = 84.2 + 0.411 x

9.4.4 Condition factor(CF)

The range of individual fish CFs was 1.49 - 2.90. Two-sample t-tests were calculated, comparing the mean CFs of pigmented and malpigmented fish in each treatment tank

at each measurement day. The null hypothesis of the tests was that there was no difference in the mean CFs of the two groups of fish. Table 9.6 summarises the results of the tests and indicates that only at day 33 did malpigmented fish have a significantly higher CF than pigmented fish. While the difference in CFs at day 33 (0.09) was significant, this difference altered little from the initial establishment of the tank fish populations (0.06) and was not evident during other measurement periods. A real difference in CF due to pigmentation was considered unlikely, so pigmented and malpigmented CFs of fish from the same tank were combined, for further analyses.

Table 9.6: Results of two-sample t-test comparing the mean CFs of pigmented and malpigmented fish (H_0: mean difference = 0)

day	pigm. type (pigm./mal)	mean CF	d.f.	t	p
0	p	1.83			
0	m	1.89	12	-1.60	0.13
14	p	1.84			
14	m	1.92	13	-1.37	0.19
33	p	1.80			
33	m	1.89	16	-2.17	**0.05**
46	p	1.80			
46	m	1.90	11	-2.08	0.06
56	p	1.80			
56	m	1.88	10	-1.27	0.23

The influence of water depth on CF was investigated using plots, correlation and regression analyses, which quantified the distribution of tank mean fish CFs with

tank water depth during the same day and within each flow type tank group during the same day. Results indicated that tank mean fish CF was not associated with water depth, at any time during the experiment. Separation of results into either those originating from tanks with the same flow rate or the same residence time, also produced no significant associations between tank mean fish CF and water depth.

No pronounced trends in the difference in distribution of tank mean fish CFs at the beginning and end of the experiment were evident (Figure 9.12). A small, and not significant, decrease in CF with increased depth at the start of the experiment was less pronounced at the end.

Results of correlation and regression analyses confirmed that individual fish CF distribution did not change significantly throughout the experiment, unlike fish weight and length. Figures 9.13 and 9.14 indicate tank mean fish CF fluctuations with time and show a negligible overall fish mean CF change from 1.85 at day 0 to 1.84 at day 56.

9.4.5 Specific growth rate

Tank mean G_Ws of fish varied between 0.71 - 1.66 % day^{-1}.

Figure 9.12: Tank mean fish CF distribution at different water depths

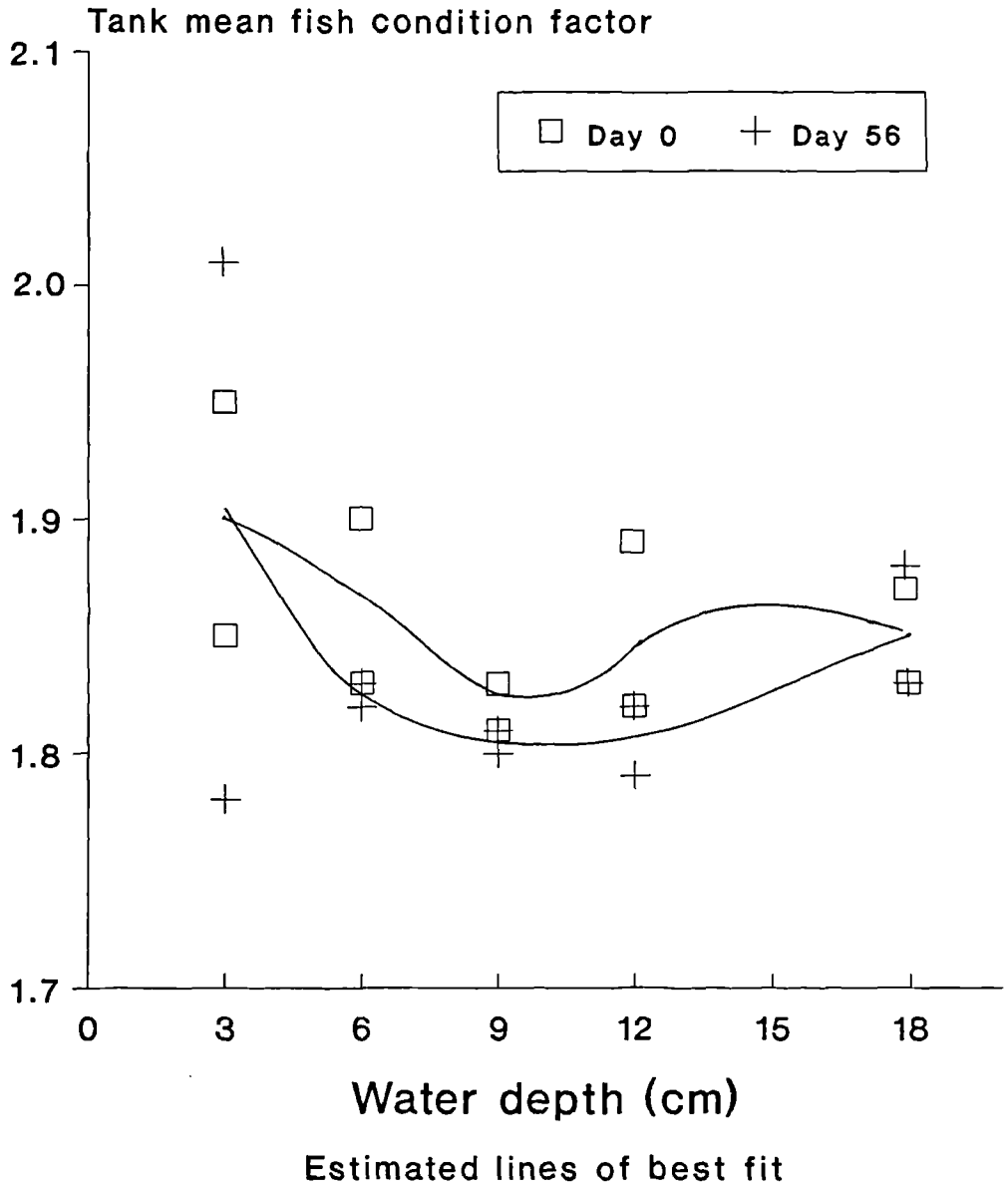
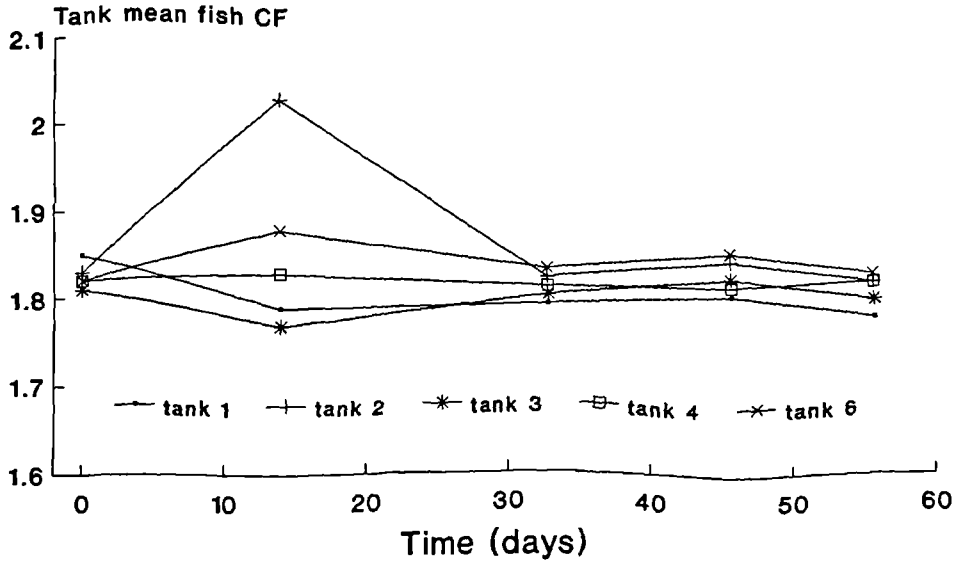
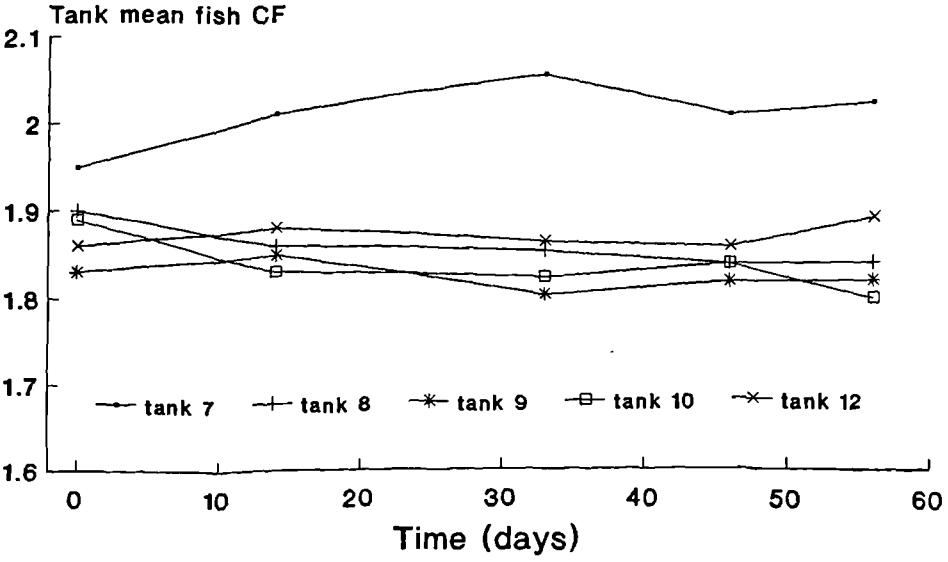


Figure 9.13: Tank mean fish CF changes with time - same residence time tanks



Water depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 6 - 18cm

Figure 9.14: Tank mean fish CF changes with time - same flow rate tanks



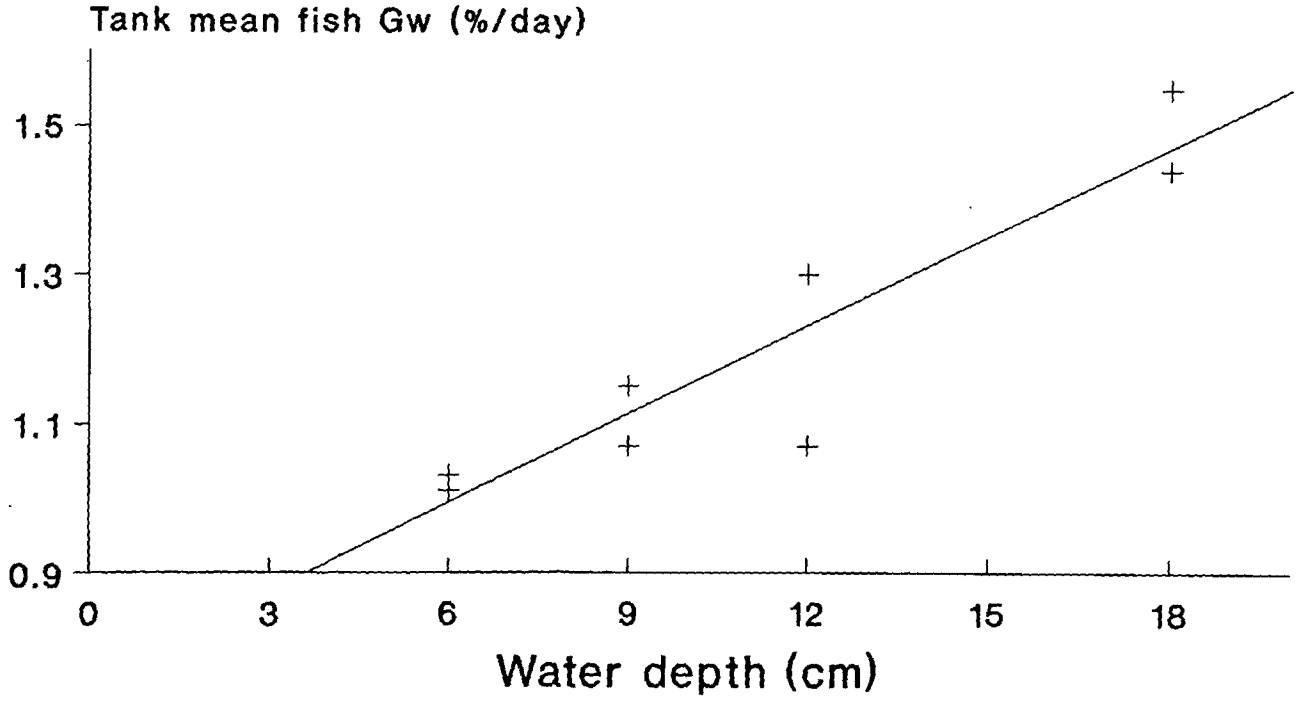
Water depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 12 - 18cm

The G_W of pigmented and malpigmented fish in the same tanks were compared during each of the four growth periods, using two-sample t-tests. Results indicated that the G_Ws of the two pigmentation groups of fish were not significantly different from each other at any time during the experiment. Analysis of variance tests, comparing the distribution of G_Ws of the pigmentation groups during each growing period, indicated that G_W was not significantly different in any of the pigmentation groups, even during different growing periods. Pigmentation was therefore discounted as a factor influencing G_W, so the G_Ws of pigmented and malpigmented fish were combined to produce tank mean G_W values for further analyses.

Results of plots, correlation and regression analyses indicated that depth did not significantly influence the tank mean G_W of fish in tanks with the same residence time, the same flow rate, or all tanks. Examination of the plots, describing the tank mean G_W distributions with respect to water depth, revealed that during the first growth period of the experiment, there was a significant association between G_W and water depth between 6 - 18 cm (Figure 9.15) when the G_Ws from all tanks were combined ($r=0.923$, $df=6$, $p<0.01$).

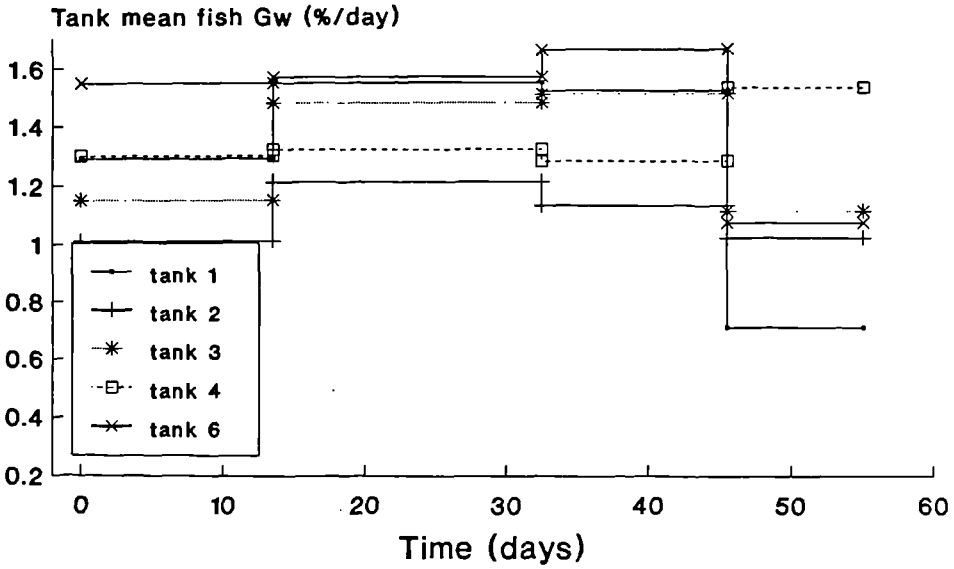
Correlation and regression analyses indicated that there was no significant linear association between G_W and time. Figures 9.16 and 9.17 indicate that fluctuations in fish G_W with time in tanks 1, 2, 3, 6, 9 and 10 were

Figure 9.15: Tank mean fish Gw in tanks with water depths between 6 - 18 cm during the period 0 - 13 days



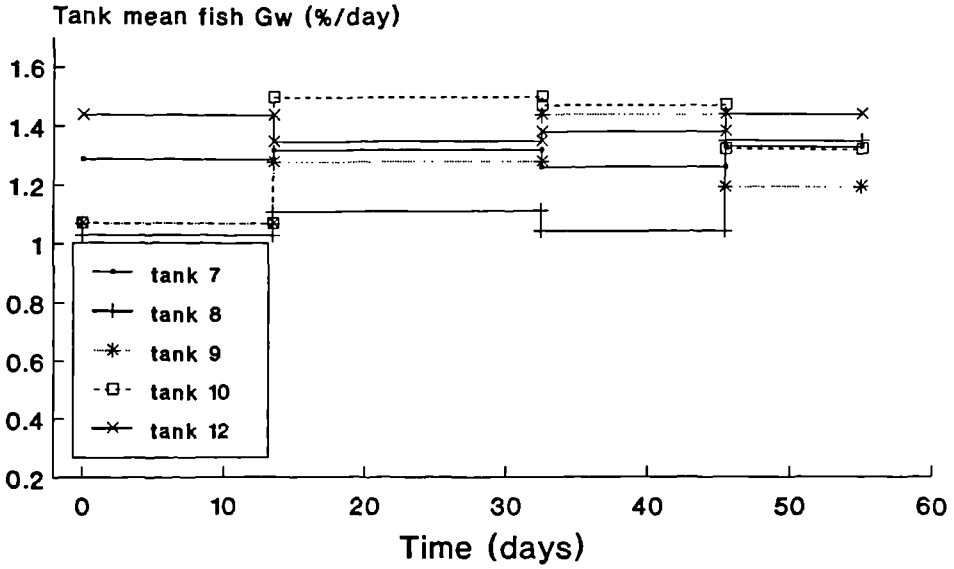
Total number of fish = 100
y = 0.756 + 0.0397 x, r = 0.923
df = 6, p < 0.001

Figure 9.16: Tank mean fish Gw changes with time - same residence time tanks



Depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 6 - 18cm

Figure 9.17: Tank mean fish Gw changes with time - same flow rate tanks



Depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 12 - 18cm

similar. G_ws rose from initial values to peak during the second or third period. This was followed by a decline until the end of the experiment.

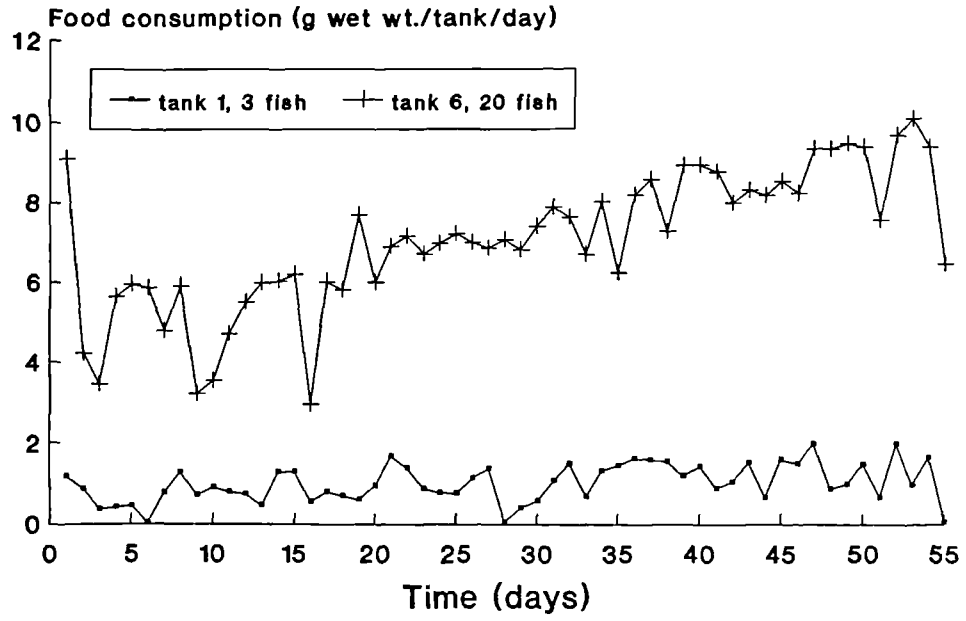
9.4.6 Food consumed

Food consumption in each tank increased during the experiment, as indicated by representative examples shown in Figures 9.18 and 9.19. Differences between daily tank mean food consumption values in each tank were related primarily to differences in the number of fish present in each tank.

The only pronounced change in food consumption with time was a decrease in food consumption in all 10 tanks between days 14 or 15 and day 16. This period was 1 - 3 days after the fish were weighed and measured, but no similar pattern was observed after any other measurement days. Coincident changes in water quality or feeding method during this period were not evident. Tank mean food consumptions during a growth period ranged between 0.09 - 3.26 % of body weight day^{-1} (wet/live). Mean consumption by all fish at day 14 was 0.32 g wet food $fish^{-1}$ day^{-1}, rising to a mean of 0.46 g wet food $fish^{-1}$ day^{-1} at day 55, equivalent to approximate means of 2.34 and 1.54 % of body weight day^{-1} (wet weight/wet weight) respectively.

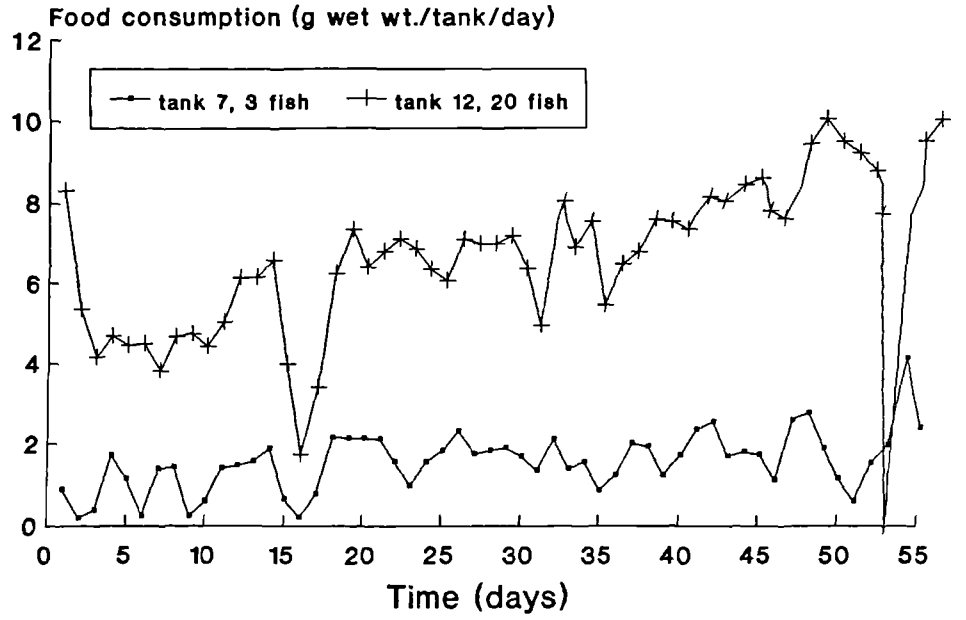
Daily food consumption fluctuations, expressed as a

Figure 9.18: Daily food consumption fluctuations - wet weight



Depths: tank 1 - 3cm, tank 6 - 18cm

Figure 9.19: Daily food consumption fluctuations - wet weight



Depths: tank 7 - 3cm, tank 12 - 18cm

percentage of body weight (wet weight /live weight), are shown in Figure 9.20. No clear associations between the percentage of body weight consumed and either treatment tank or time, were detected.

9.4.7 Food conversion ratio

Wet feed weight FCRs ranged between 1.18 - 2.56, while dry feed weight FCRs ranged between 0.69 - 1.50.

Table 9.7 summarizes the results of correlation and regression analyses calculated to investigate the influence of water depth on FCR of fish in tanks with the same residence times, the same flow rates, or all tanks studied. The results (Figures 9.21 and 9.22) showed a significant linear decline in FCR, and therefore a decrease in the weight of food required to increase weight, with increased water depth, between days 33 - 45. This association was particularly evident at water depths of 6 - 18 cm, but fish in the 3 cm deep tanks did not exhibit a FCR as high as that suggested by the linear regression equation.

No trends in FCR fluctuations with respect to time were evident, other than a marginal decrease, ie. improvement, in FCR throughout the experiment in tanks with the same flow rates.

Figure 9.20: Tank mean wet weight of food consumed expressed as a proportion of the fish wet weight

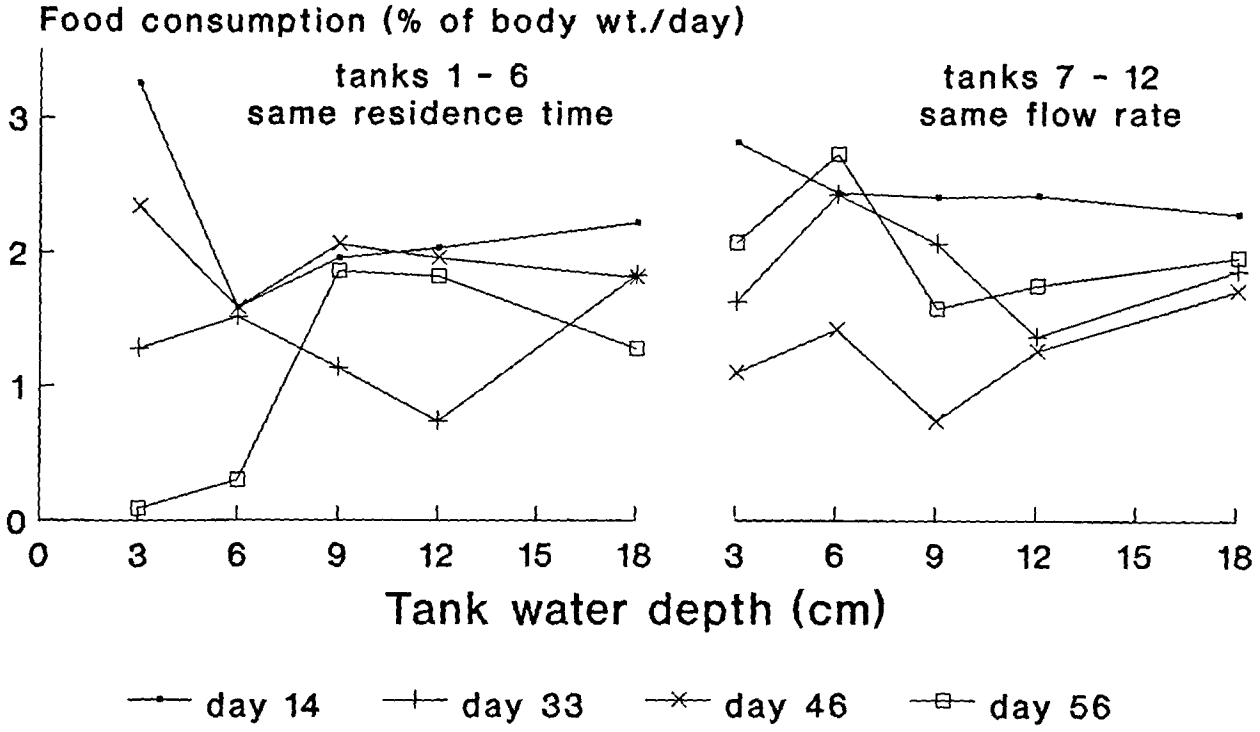
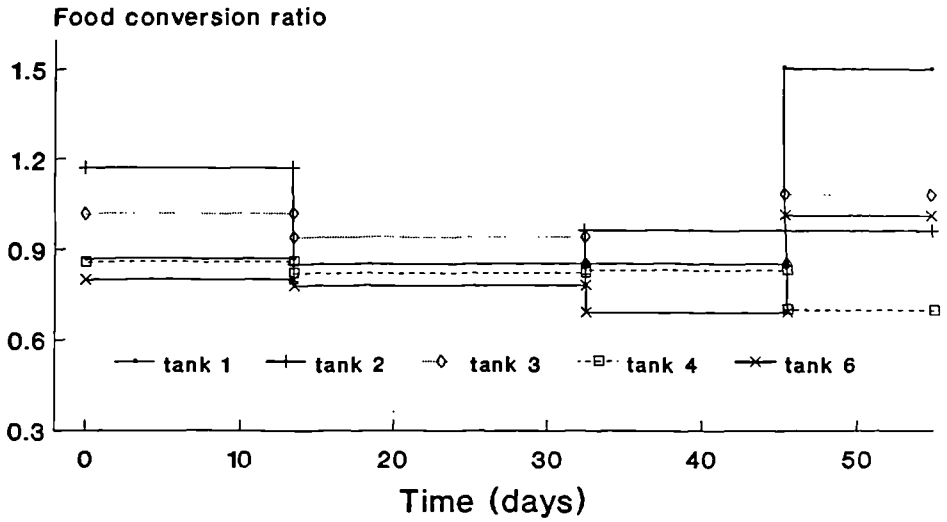
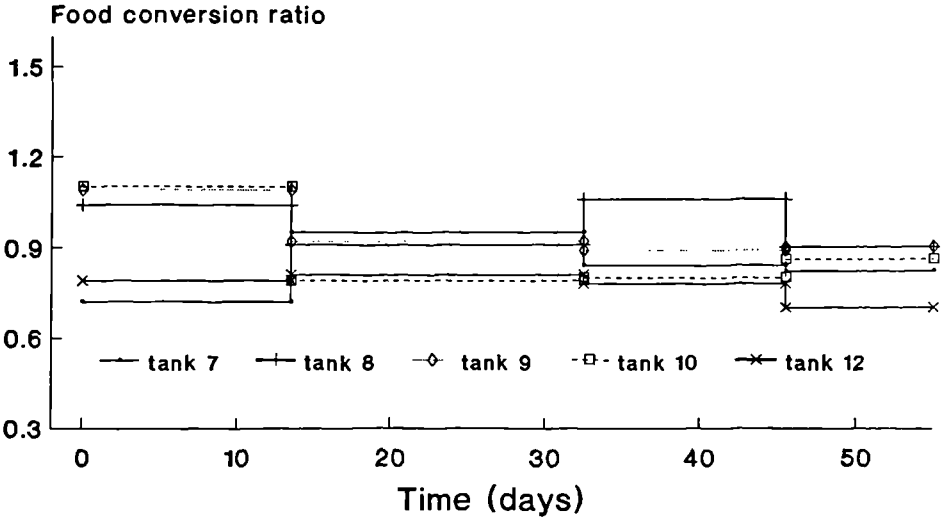


Figure 9.21: Fluctuations of FCR (dry feed/live weight) with time same residence time tanks



Depths: tank 1 - 3cm, tank 2 - 6cm
tank 3 - 9cm, tank 4 - 12cm
tank 6 - 18cm

Figure 9.22: Fluctuations of FCR (dry feed/live weight) with time same flow rate tanks



Depths: tank 7 - 3cm, tank 8 - 6cm
tank 9 - 9cm, tank 10 - 12cm
tank 12 - 18cm

Table 9.7: Summary of results of analyses investigating the influence of water depth on tank mean fish food conversion ratio (dry feed/live weight)

Growth period (days)	tank group	d.f.	r	regression p
0 - 13	r	3	-0.514	0.375
0 - 13	f	3	0.030	0.961
0 - 13	all	8	-0.217	0.547
14 - 32	r	3	-0.165	0.791
14 - 32	f	3	-0.850	0.068
14 - 32	all	8	-0.492	0.149
33 - 45	r	3	-0.804	0.101
33 - 45	f	3	-0.549	0.338
33 - 45	all	8	-0.652	**0.041**
46 - 55	r	3	-0.574	0.311
46 - 55	f	3	-0.652	0.233
46 - 55	all	8	-0.451	0.191

r = same residence time tanks
f = same flow rate tanks

9.4.8 Daily water quality fluctuations

Results of water quality samples, taken at 11.30 h daily throughout the experiment (days 0 - 56), were used to examine daily changes in recirculation system water quality.

Temperature

Recirculation system water temperature varied between 14.8 - 17.4 ^{o}C with a mean of 16.2 ^{o}C (Figure 9.23). A marked weekly periodicity in temperature fluctuation was

apparent. Seven of the eight falls in temperature occurred on either a Sunday or Monday.

pH

Recirculation system pH varied between 7.27 - 7.78 (Figure 9.24). A steady fall in pH between days 1 - 39 was arrested by the occasional addition of sodium hydrogen carbonate buffer, as described in section 4.2.2, which was used to restore a pH of approximately 7.7 - 7.8.

Salinity

Salinity, which varied between 34.5 - 35.4 ppt (Figure 9.24), was maintained approximately constant at 35 ppt throughout the experiment by the addition of calculated quantities of dechlorinated freshwater, irregularly as required.

Dissolved oxygen

DO concentrations varied between 7.1 - 8.1 mg l^{-1} (Figure 9.23), with a mean of 7.5 mg l^{-1}, equivalent to variations between 90.2 - 99.5 % saturation with a mean of 94.2 %.

9.4.9 24 h water quality fluctuations

Water quality in tanks 1, 3, 6, 7, 9 and 12 was

Figure 9.23: Recycle system temperature and DO fluctuations

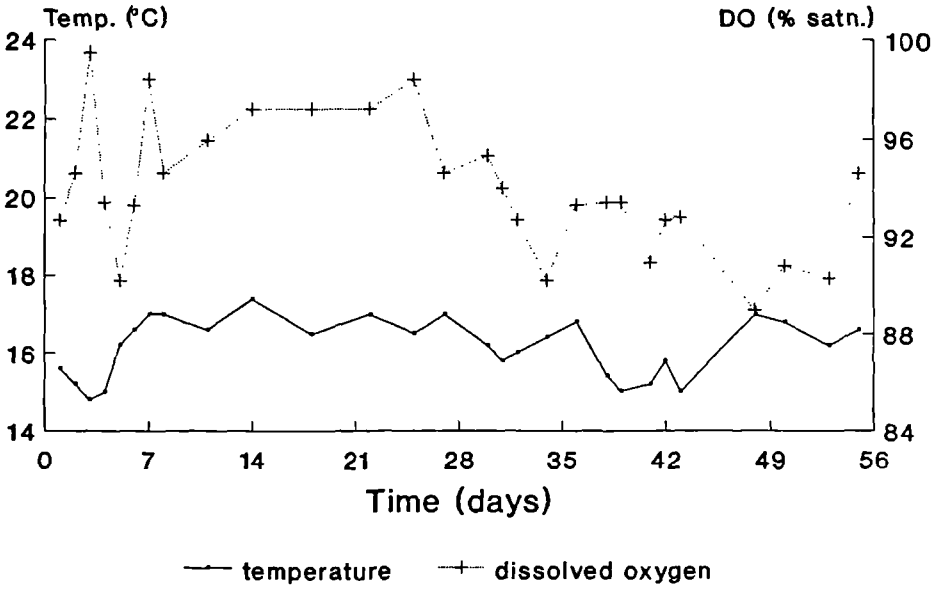
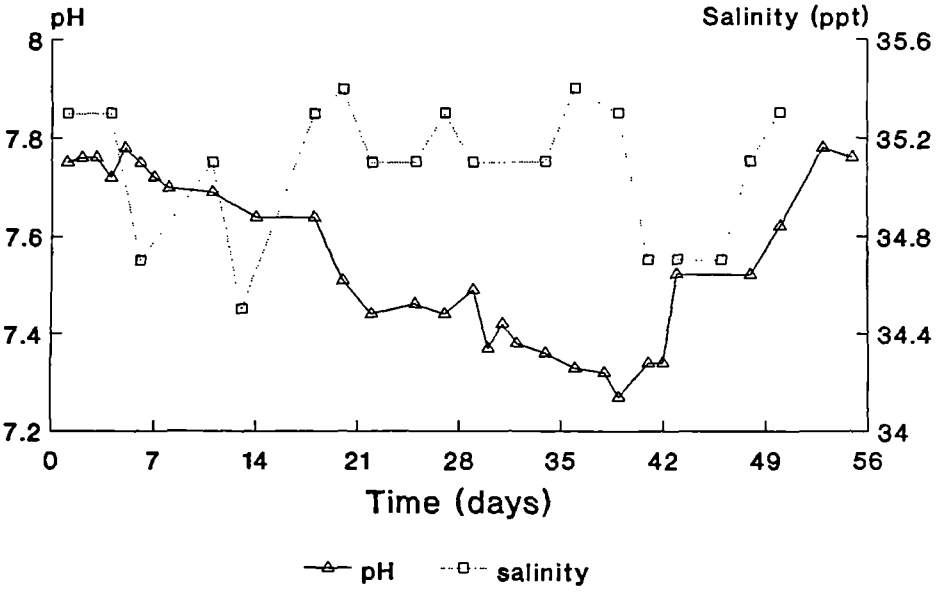


Figure 9.24: Recycle system pH and salinity fluctuations



investigated, using the schedule described in section 8.3.6 and analytical techniques described in section 4.6, on days 52 - 53.

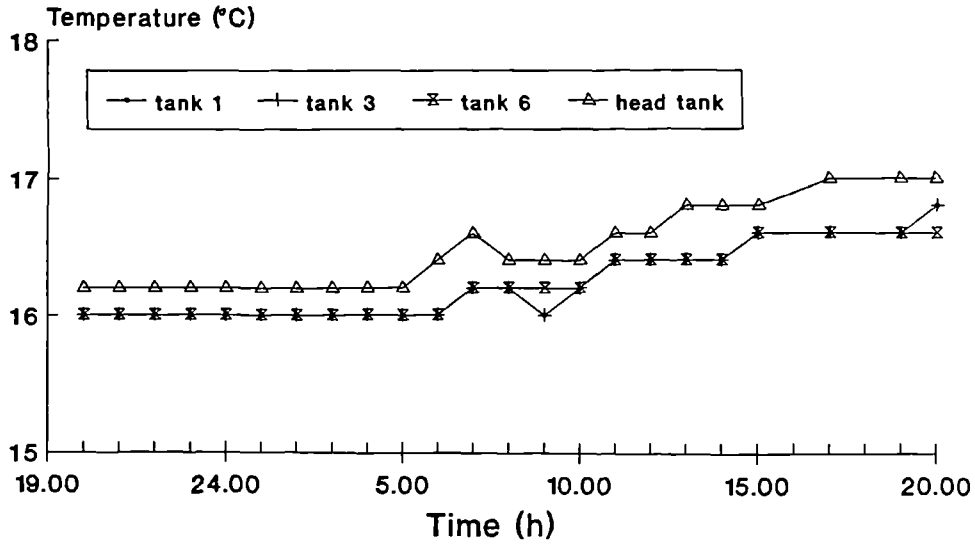
Temperature

Treatment tank water temperature increased during the sample period from approximately 16.0 - 16.6 ^{o}C and fluctuated by a maximum of 0.2 ^{o}C between each hourly sample (Figures 9.25 and 9.26). Water temperatures appeared to follow air temperatures with a lag of 2 h. Temperature fluctuations were similar in all treatment tanks, so no influence of water depth on water temperature was apparent. Head tank water temperatures were usually 0.2 ^{o}C higher than the treatment tanks. Overnight, between approximately 20.00 - 05.00 h, water temperatures remained constant. A rise and fluctuations in temperature occurred between approximately 07.00 - 16.00 h, coincident with increased air temperatures.

pH

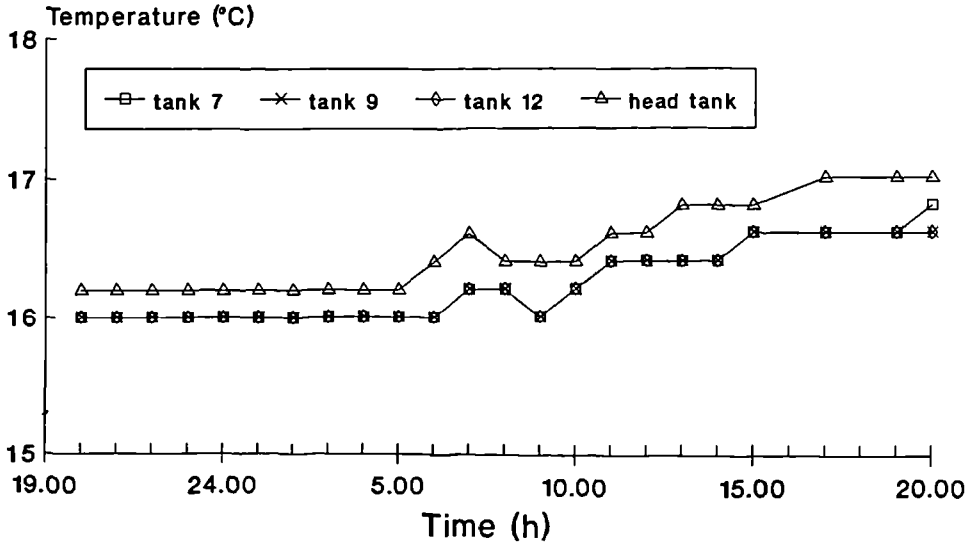
pH in all treatment tanks and the head tank water followed a similar pattern of fluctuations (Figures 9.27 and 9.28). Two distinct periods of change were apparent. Between 20.00 h and approximately 08.00 h, pH increased. Between 08.00 - 20.00 h, pH declined. The most pronounced period of decline began within approximately 0.5 h after feeding and lasted about 4 h, to 17.00 h. Overall, pH

Figure 9.25: Water temperature fluctuations during a 24 h period - same residence time tanks



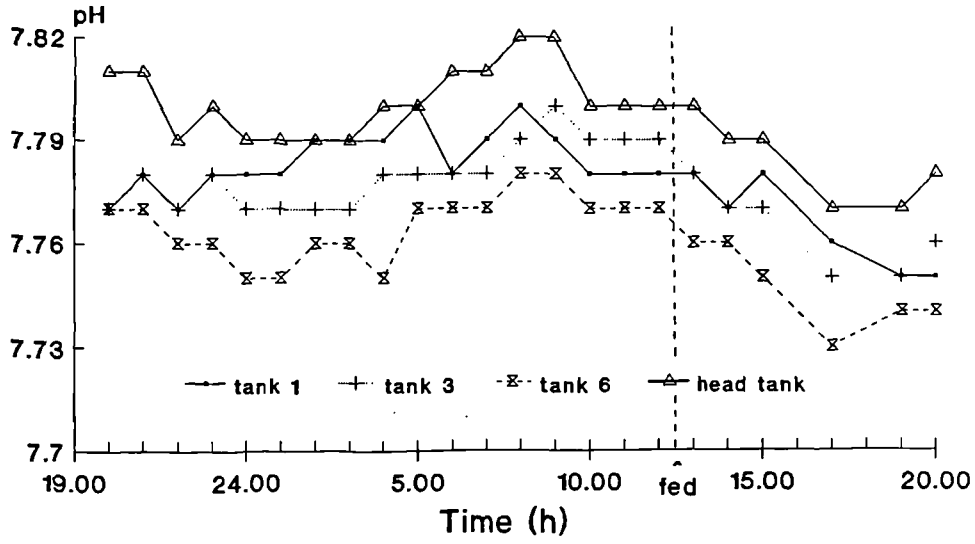
Depths: tank 1 - 3cm, tank 3 - 9cm
tank 6 - 18cm

Figure 9.26: Water temperature fluctuations during a 24 h period - same flow rate tanks



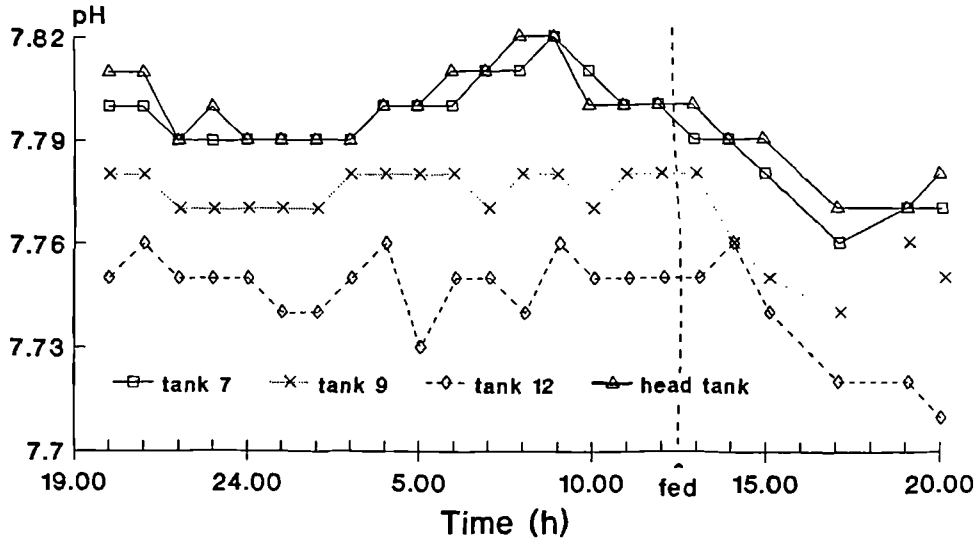
Depths: tank 7 - 3cm, tank 9 - 9cm
tank 12 - 18cm

Figure 9.27: Water pH fluctuations during a 24 h period - same residence time tanks



Depths: tank 1 - 3cm, tank 3 - 9cm
tank 6 - 18cm

Figure 9.28: Water pH fluctuations during a 24 h period - same flow rate tanks



Depths: tank 7 - 3cm, tank 9 - 9cm
tank 12 - 18cm

decreased during the 24 h period from 7.75 - 7.81 to 7.71 - 7.78, a fall of approximately pH 0.03 day^{-1}. Water in tanks with the same residence time (1, 3 and 6) appeared less susceptible to fluctuations in pH than water in tanks with the same flow rate (7, 9 and 12).

During 22 of the 23 pH sample times, the head tank (influent water) pH was greater than, or equal to, the pH in any of the treatment tanks. The maximum difference between influent and treatment tank water pH was 0.06. Table 9.8 indicates the mean of the differences in pH between each treatment tank and the influent water, at each sample time. All tanks contained water of a significantly distinct pH, as indicated by paired-sample t-tests. Results (Figure 9.29; Table 9.8) indicated that at any particular time the likely order, from highest to lowest pH, of tanks in which pH was significantly different was: head tank/influent > 7 > 1 > 3 > 9 > 6 > 12. This sequence indicates an association between pH and depth which was analysed further by correlation and regression analyses quantifying the influence of depth on the drop in pH between the influent and treatment tanks sample points. In tanks with the same flow rate, an increase in the pH drop between the influent and the treatment tank was significantly associated with an increase in treatment tank water depth ($r=0.998$, $df=1$, $p<0.05$). On combining results (Figure 9.29) a similar significant association was evident ($r=0.908$, $df=4$, $p<0.05$).

Table 9.8: Mean pH differences during a 24 h period (tanks a pH - tanks b pH)

tanks (a)		1	3	6	7	9	12	head
depth (cm)		3	9	18	3	9	18	
	1	-	-0.003	-0.019	0.014	-0.008	-0.035	0.018
	3	0.003	-	-0.015	0.018	-0.005	-0.032	0.021
Tanks	6	0.019	0.015	-	0.033	0.010	-0.017	0.037
(b)	7	-0.014	-0.018	-0.033	-	-0.023	-0.050	0.003
	9	0.008	0.005	-0.010	0.023	-	-0.027	0.026
	12	0.035	0.032	0.017	0.050	0.027	-	0.053
	head	-0.018	-0.021	-0.037	-0.003	-0.026	-0.053	-

All differences were statistically significant ($p<0.05$).

Salinity

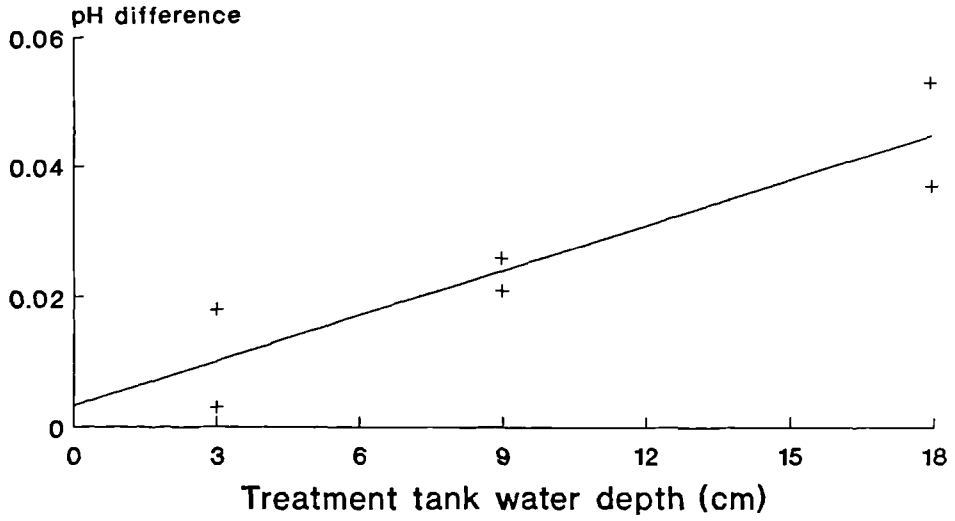
A constant salinity of 35.3 ppt was maintained, with no detectable fluctuations during the sample period.

Dissolved oxygen

DO concentration during the 24 h sample period ranged between 6.3 - 7.7 mg l^{-1}, corresponding to 79.0-96.5 % saturation (Figures 9.30 and 9.31). No obvious patterns in DO saturation with respect to the time of day or feeding were evident, other than a fall in DO saturation between 04.00 - 06.00 h in most tanks. Hourly fluctuations were small and rarely exceeded 5 % saturation.

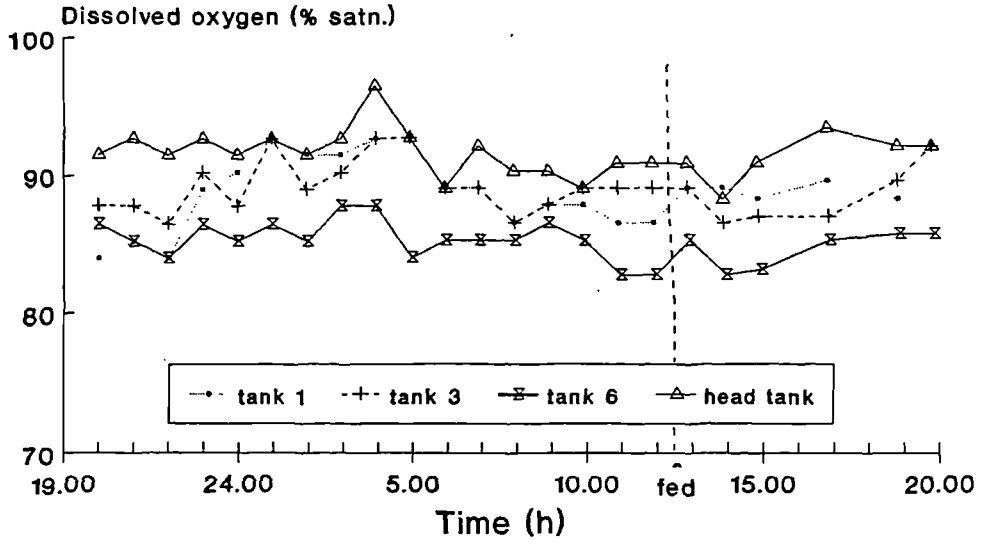
Table 9.9 indicates the mean DO differences between each of the sample points throughout the 24 h period.

Figure 9.29: Influence of water depth on the pH difference between water from the influent and treatment tanks



Head tank pH - treatment tank pH
y = 0.00357 + 0.00228 x, r = 0.908
df = 4, p <0.05

Figure 9.30: DO concentration changes during a 24 h period - same residence time tanks



Depths: tank 1 - 3cm, tank 3 - 9cm
tank 6 - 18cm

Most tanks contained water with a significant mean difference in DO percentage saturation. The following sequence of tanks in order of increasing DO saturation difference between the treatment tanks and the influent (head tank) was evident from the mean results: head tank < 7 < 3 < 1 < 9 < 6 < 12. The magnitude of the drop was significantly associated with water depth in tanks with the same flow rate ($r=1.000$, $df=1$, $p<0.05$), in all tanks tested ($r=0.929$, $df=4$, $p<0.01$, Figure 9.32), but not in tanks with the same residence time. The decrease in DO between the head tank and the treatment tank centres therefore increased significantly with increased water depth.

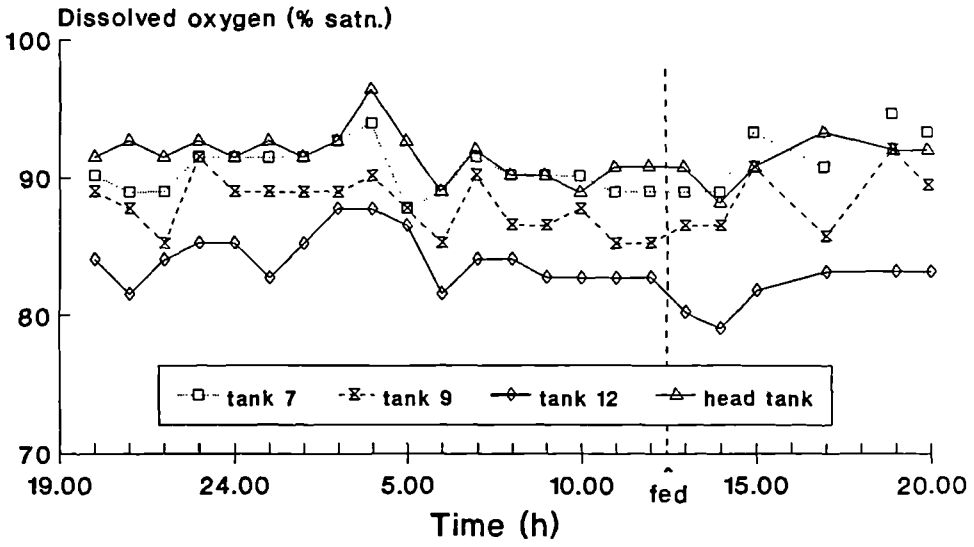
Table 9.9: Mean dissolved oxygen (% saturation) differences during a 24 h period (tanks a DO - tanks b DO)

tanks (a)		1	3	6	7	9	12	head
depth (cm)		3	9	18	3	9	18	
	1	-	0.22	**-3.63**	**1.97**	-0.76	**-5.30**	**2.72**
	3	-0.22	-	**-3.85**	**1.75**	-0.97	**-5.52**	**2.50**
tanks	6	**3.63**	**3.85**	-	**5.60**	**2.87**	**-1.67**	**6.35**
(b)	7	**-1.97**	**-1.75**	**-5.60**	-	**-2.73**	**-7.27**	0.75
	9	0.76	0.97	**-2.87**	**2.73**	-	**-4.55**	**3.48**
	12	**5.30**	**5.52**	**1.67**	**7.27**	**4.55**	-	**8.03**
	head	**-2.72**	**-2.50**	**-6.35**	-0.75	**-3.48**	**-8.03**	-

Total ammonia

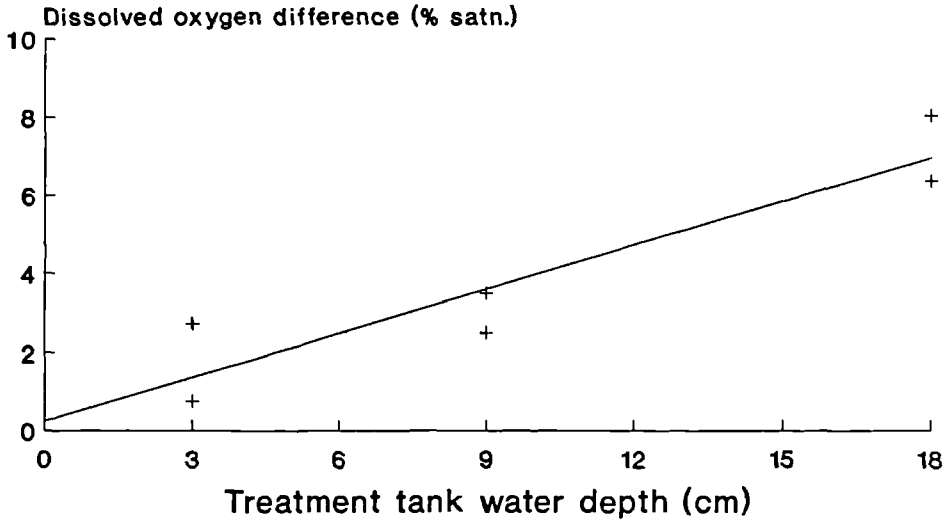
NH_4-N values fluctuated between 0.019 - 0.133 mg NH_4-N l^{-1} during the 24 h sample period (Figures 9.33 and 9.34). Within this range, fluctuations between consecutive samples were large, particularly in tanks with

Figure 9.31: DO concentration changes during a 24 h period - same flow rate tanks



Depths: tank 7 - 3cm, tank 9 - 12cm
tank 12 - 18cm

Figure 9.32: Influence of water depth on the DO difference between water from the influent and treatment tanks



Head tank DO - treatment tank DO
y = 0.254 + 0.372 x, r = 0.929
df = 4, p <0.01

Figure 9.33: Total ammonia concentration changes during a 24 h period - same residence time tanks

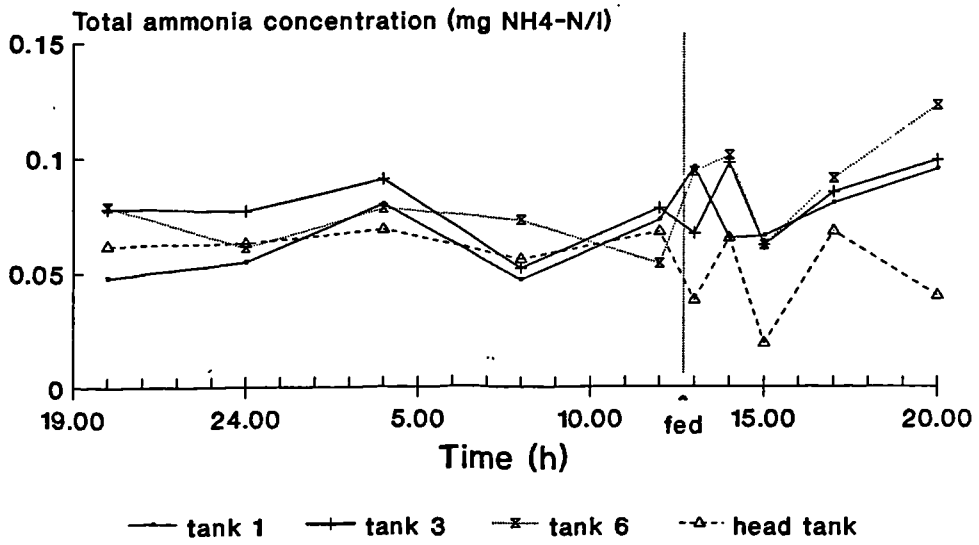
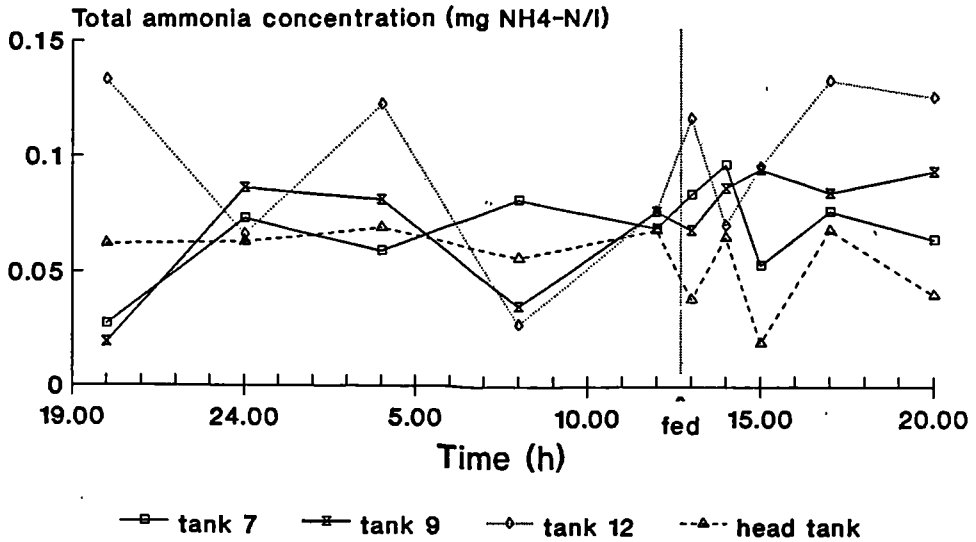


Figure 9.34: Total ammonia concentration changes during a 24 h period - same flow rate tanks



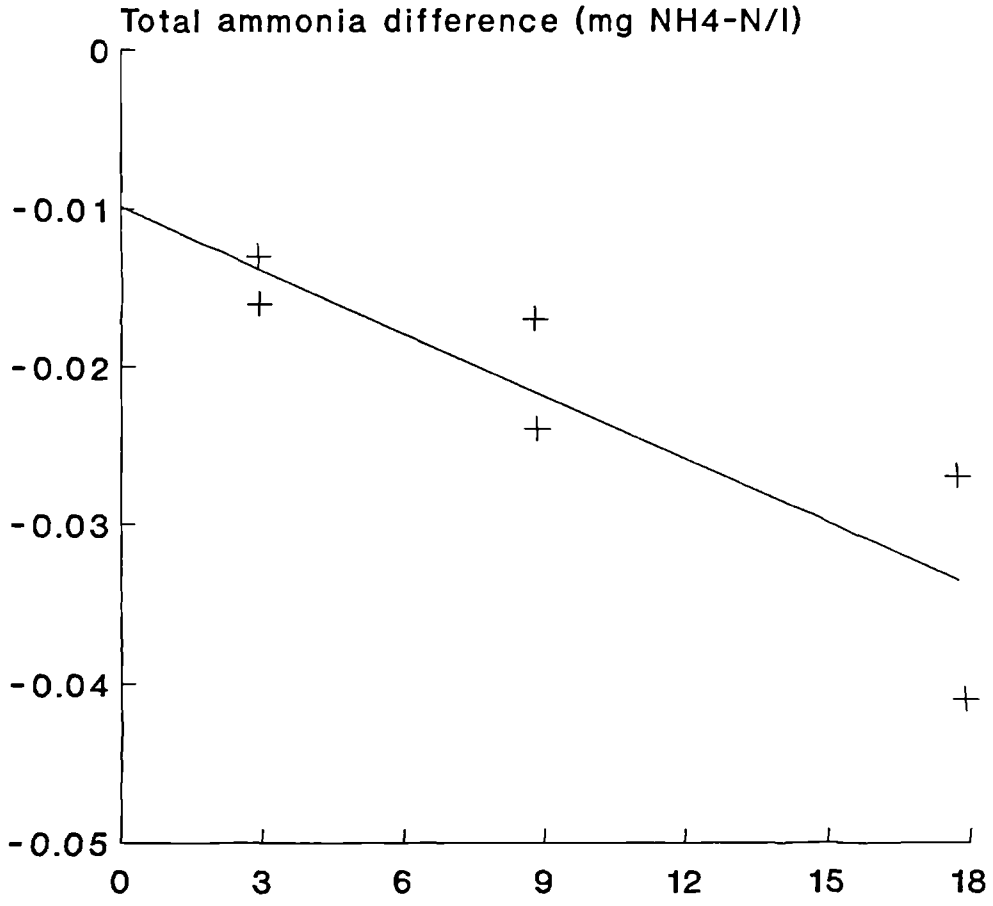
the same flow rate. Despite this variability, a diurnal cycle of fluctuations was apparent. NH_4-N concentrations were fairly constant, or decreased slightly overnight. This was followed by rises, from approximately 2.5 h after feeding, to a peak at approximately 20.00 h, 7.5 h after feeding.

Table 9.10 summarizes the mean differences in NH_4-N concentrations between the sample points. The following sequence of tanks in order of increasing NH_4-N concentrations was evident from the mean results: head tank < 7 < 1 < 9 < 3 < 6 < 12. A clear association between depth and total ammonia concentration is evident in this sequence. A plot (Figure 9.35) of treatment tank concentration relative to the influent concentration (head tank) illustrates this significant association. Total ammonia concentration increase between the influent and treatment tanks was greater in the deeper tanks ($r=0.866$, $df=4$, $p<0.05$).

Table 9.10: Mean total ammonia (mg NH_4-N l^{-1}) differences during a 24 h period (tanks a NH_4-N - tanks b NH_4-N)

tanks (a)		1	3	6	7	9	12	head
depth (cm)		3	9	18	3	9	18	
	1	-	0.008	0.011	-0.002	0.002	**0.026**	-0.016
	3	-0.008	-	0.003	-0.011	0.007	0.018	**-0.024**
Tanks	6	-0.011	-0.003	-	-0.014	-0.009	0.015	**-0.027**
(b)	7	0.002	0.011	0.014	-	0.004	0.028	-0.013
	9	-0.002	0.007	0.009	-0.004	-	0.024	-0.017
	12	**-0.026**	-0.018	-0.015	-0.028	-0.024	-	**-0.041**
	head	0.016	**0.024**	**0.027**	0.013	0.017	**0.041**	-

Figure 9.35: Total ammonia concentration difference between influent and treatment tank water



Head tank - treatment tank
y = 0.00984 + 0.00132 x, r = 0.866
df = 4, p <0.05

Unionized ammonia

NH_3-N fluctuations (Figures 9.36 and 9.37) were similar to total ammonia fluctuations described above, concentrations varying between 0.234 - 1.463 /ug NH_3-N l^{-1}.

Table 9.11 summarizes the mean differences in NH_3-N concentrations between the sample points. Influent concentrations were significantly lower than tanks 3, 7, 9 and 12. The following sequence of tanks in order of increased unionized ammonia concentrations, relative to the influent (head tank), was evident from the mean results: head tank < 7 < 1 < 6 < 9 < 3 < 12. An association between depth and NH_3-N concentration, similar to that described for NH_4-N was evident in this sequence, but was less pronounced and was not significant, due mainly to the higher concentration of NH_3-N relative to the head tank which occurred in tank 6, compared with NH_4-N results.

Table 9.11: Mean unionized ammonia (/ug NH_3-N l^{-1}) differences during a 24 h period (tanks a NH_3-N - tanks b NH_3-N)

tanks (a)		1	3	6	7	9	12	head
depth (cm)		3	9	18	3	9	18	
	1	-	0.073	0.019	-0.010	0.045	0.197	-0.188
	3	-0.073	-	-0.054	-0.084	-0.028	0.124	**-0.261**
Tanks	6	-0.019	0.054	-	-0.029	0.026	0.178	-0.207
(b)	7	0.010	0.084	0.029	-	0.055	0.207	**-0.178**
	9	-0.045	0.028	-0.026	-0.055	-	0.152	**-0.233**
	12	-0.197	-0.124	-0.178	-0.207	-0.152	-	**-0.385**
	head	0.188	**0.261**	0.207	**0.178**	**0.233**	**0.385**	-

Figure 9.36: Unionized ammonia concentration changes during a 24 h period - same residence time tanks

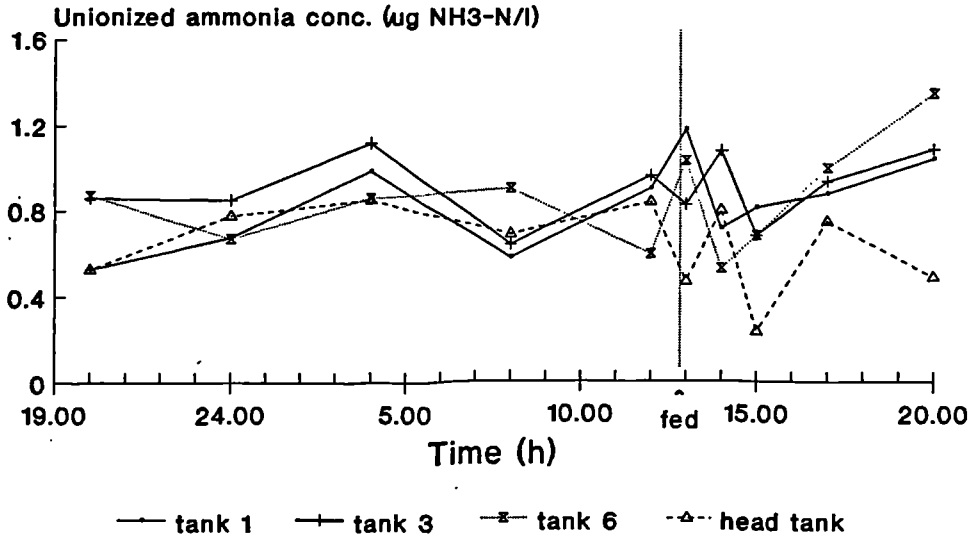
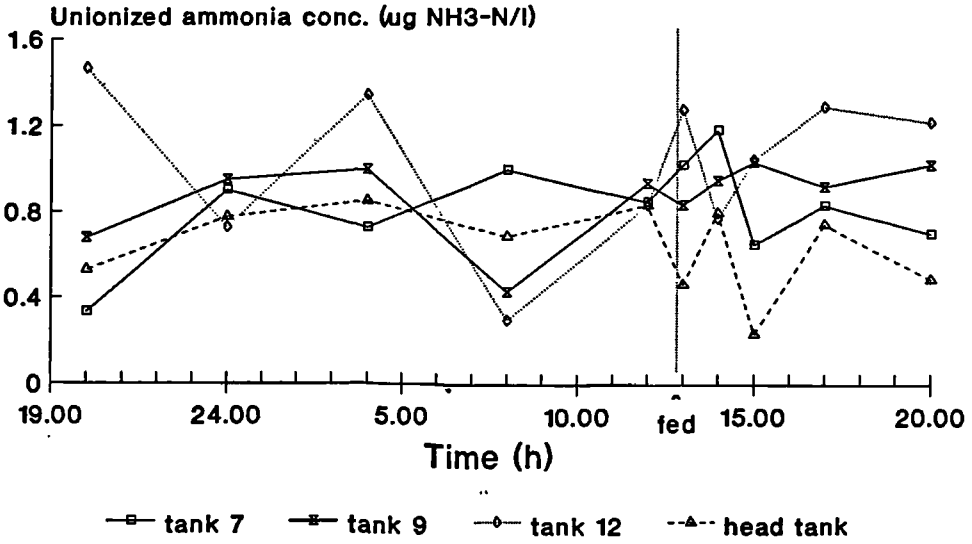


Figure 9.37: Unionized ammonia concentration changes during a 24 h period - same flow rate tanks



Nitrite

NO_2-N concentrations, shown in Figures 9.38 and 9.39, fluctuated between 0.034 - 0.064 mg NO_2-N l^{-1}.

The mean differences in nitrite concentration between the sample points at each time of sampling is summarized in Table 9.12. Differences were small and few tanks were significantly different to any others. The following sequence of tanks in order of increased nitrite concentrations, relative to the influent, was evident from the mean results: 6 < 3 < head tank < 7 < 1 < 9 < 12. No association between depth and nitrite concentration was therefore evident in this sequence.

Table 9.12: Mean nitrite (mg NO_2-N l^{-1}) differences during a 24 h period (tanks a NO_2-N - tanks b NO_2-N)

tanks (a)		1	3	6	7	9	12	head
depth (cm)		3	9	18	3	9	18	
	1	-	-0.001	-0.003	0.000	0.000	0.001	-0.001
	3	0.001	-	-0.002	0.001	0.001	0.002	0.000
Tanks	6	0.003	0.002	-	**0.002**	0.003	**0.003**	0.002
(b)	7	0.000	-0.001	**-0.002**	-	0.001	0.001	-0.001
	9	0.000	-0.001	-0.003	-0.001	-	0.000	-0.001
	12	-0.001	-0.002	**-0.003**	-0.001	0.000	-	-0.001
	head	0.001	0.000	-0.002	0.001	0.001	0.001	-

9.4.10 Within tank water quality

Results of water quality samples taken at 11.30 h on 18.3.88 (day 43) were used to examine changes in the quality of water passing from the influents, through the

Figure 9.38: Nitrite concentration changes during a 24 h period - same residence time tanks

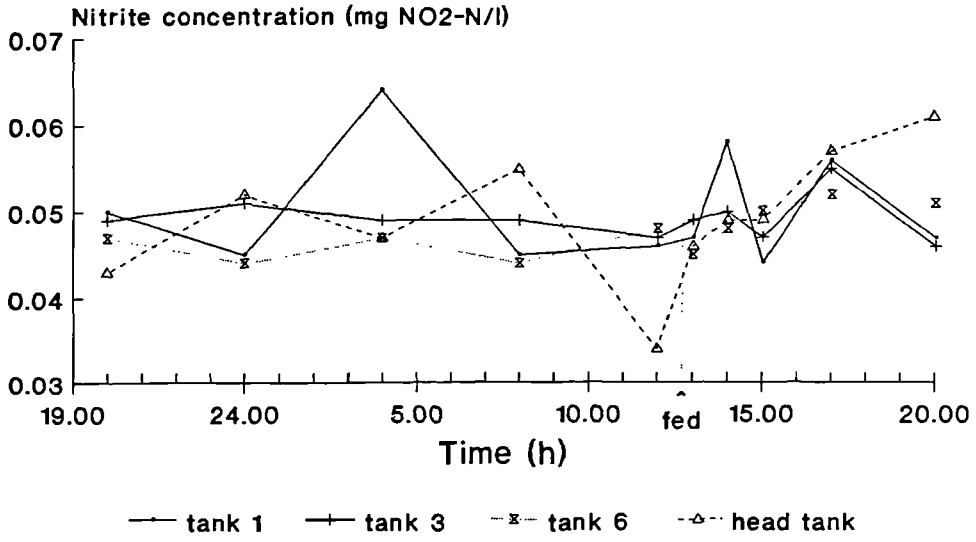
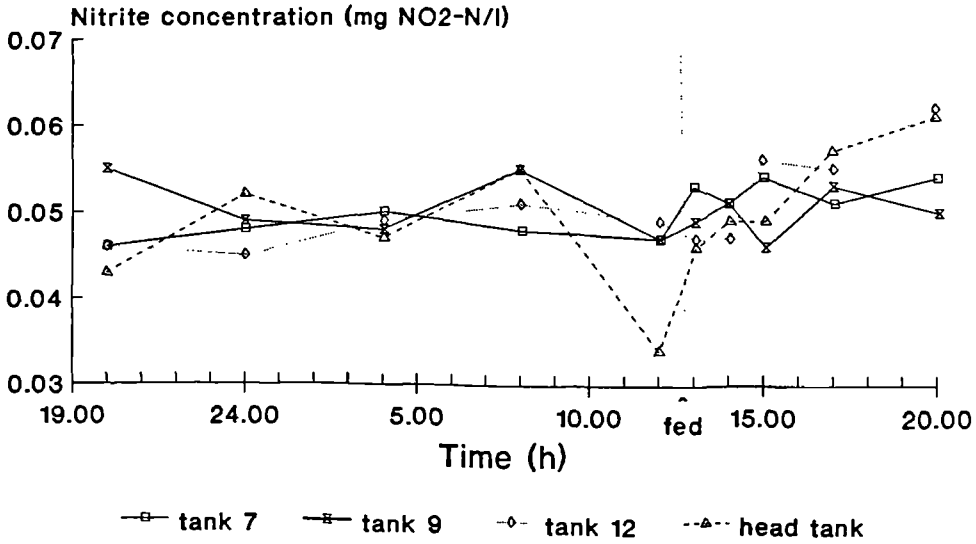


Figure 9.39: Nitrite concentration changes during a 24 h period - same flow rate tanks



treatment tanks, to the effluents.

Temperature

The temperature of all samples was 15.0 ^{o}C.

pH

pH of the samples varied within the range 7.49 - 7.51 (Figure 9.40), decreasing significantly between the influent and the tank centres (mean=0.054, t=15.89, n=10, $p<0.001$) and between the tank centres and the corresponding effluents (mean=0.011, t=3.16, n=10, $p<0.05$). The magnitude of the decreases were not significantly related to depth in the tanks with the same residence time, but pH drop between the influent and the tank centre and the influent and the tank effluents increased significantly with increased depth in tanks with the same flow rate ($r=0.955$, df=3, $p<0.05$ and $r=0.907$, df=3, $p<0.05$, respectively). This confirms results presented in section 9.4.10.

Salinity

The salinity of all samples was 34.7 ppt.

Dissolved oxygen

The DO concentration of the samples varied within the

range 7.3 - 7.7 mg l^{-1}, equivalent to 89.7 - 94.6 % saturation (Figure 9.41). The DO saturation of the treatment tank influent water was greater than any of the tank centre and effluent saturations, indicating a drop in DO as water passed through the tank. The mean DO saturation in the tank centres was not significantly different to the mean DO saturation in the corresponding effluents. The influent (head tank) and both tank centres and tank effluents DO saturations were significantly different (mean difference=2.92 %, t=8.74, n=10, $p<0.001$ and mean difference=3.03 %, t=8.99, n=10, $p<0.001$, respectively). The decrease in DO saturation therefore occurred primarily between the head tank and the treatment tank centres.

Only in tanks with the same flow rate was there any association between DO saturation drop from the head tank to either tank centres or effluents. DO drop between the head tank and the tank centres increased significantly with increased depth ($r=0.923$, $df=3$, $p<0.05$).

Total ammonia

NH_4-N concentrations of the samples varied between 0.072 - 0.141 mg NH_4-N l^{-1} (Figure 9.42). No clear trends in NH_4-N concentration with respect to the location of the sample point or to tank water depth were evident.

Figure 9.40: pH changes across the treatment tanks

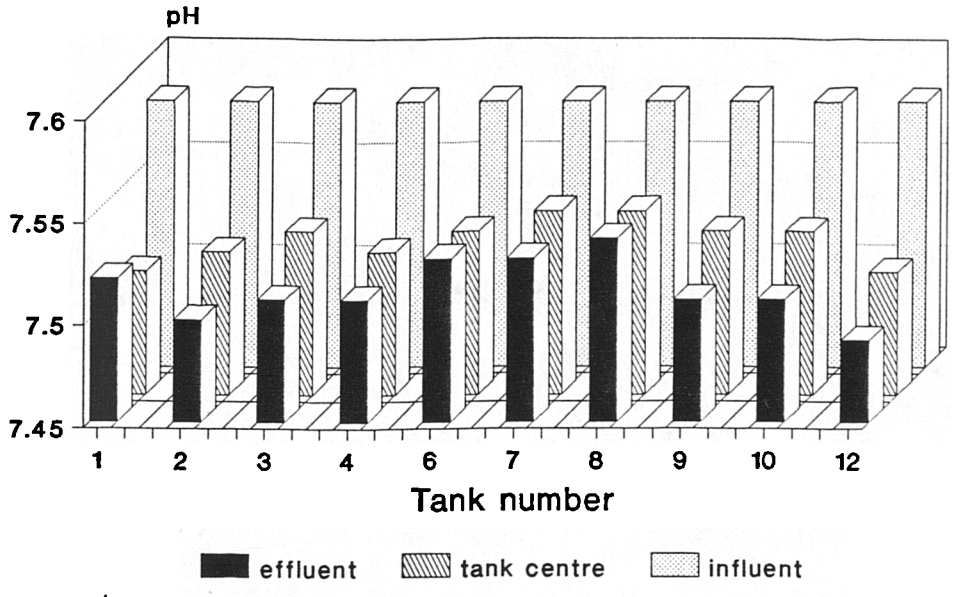
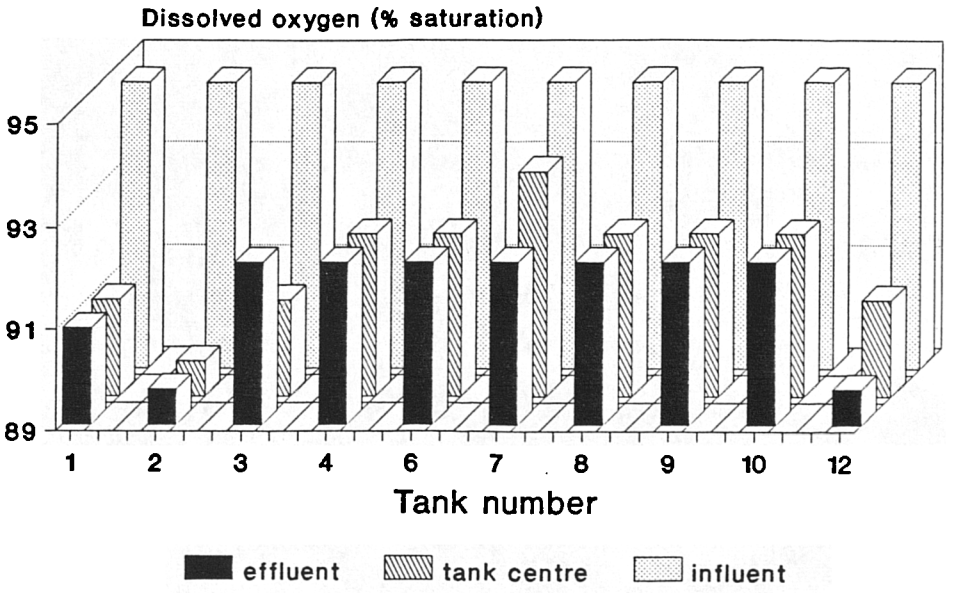


Figure 9.41: Dissolved oxygen changes across the treatment tanks



Unionized ammonia

NH_3-N concentrations ranged between 0.463 - 0.808 µg NH_3-N l^{-1} (Figure 9.43). No significant associations between NH_3-N concentration and either the location of the sample or tank water depth were determined, but the influent concentrations were greater than either tank centre or effluent concentrations in the majority of tanks.

Nitrite

NO_2-N concentrations ranged between 0.049 - 0.052 mg NO_2-N l^{-1} (Figure 9.44). No associations between nitrite concentration and either sample point location or tank water depth were evident.

9.4.11 Summary of main results

1. Survival: no fish died during the experiment.
2. Weight: fish weighed between 4.1 - 30.9 g at day 0 to 6.2 - 62.4 g at day 56.
3. Weight: tank mean fish weight distribution was not associated with fish pigmentation.
4. Weight: tank mean fish weight distribution was not associated with water depth in the treatment tanks.
5. Weight: the weight of fish in all tanks increased significantly throughout the experiment.

Figure 9.42: Total ammonia changes across the treatment tanks

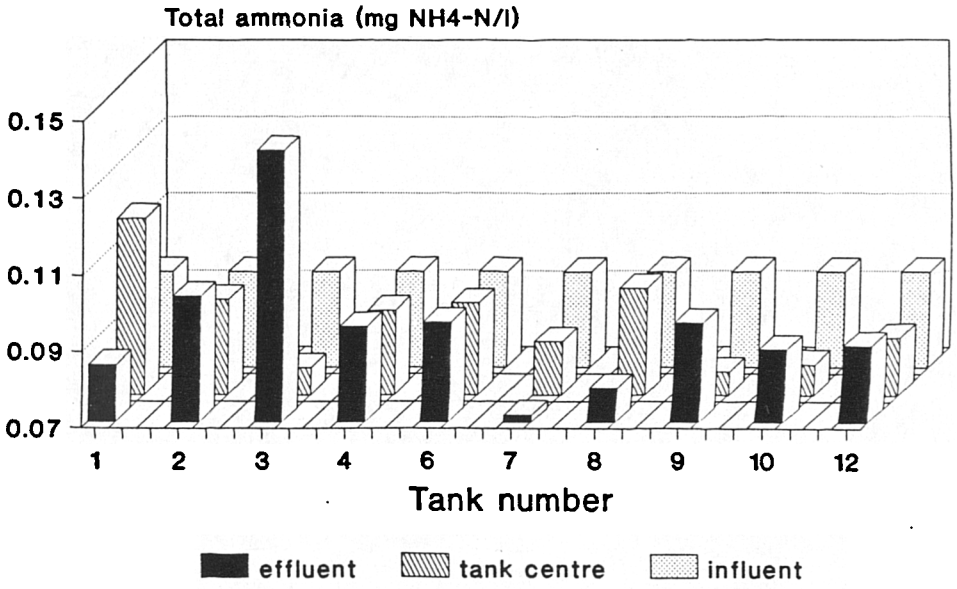


Figure 9.43: Unionized ammonia changes across the treatment tanks

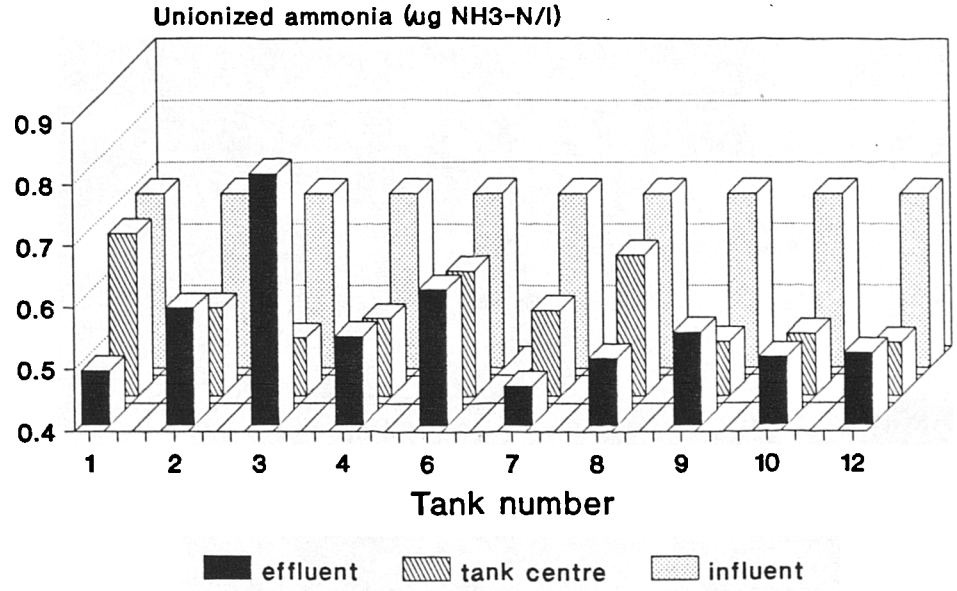
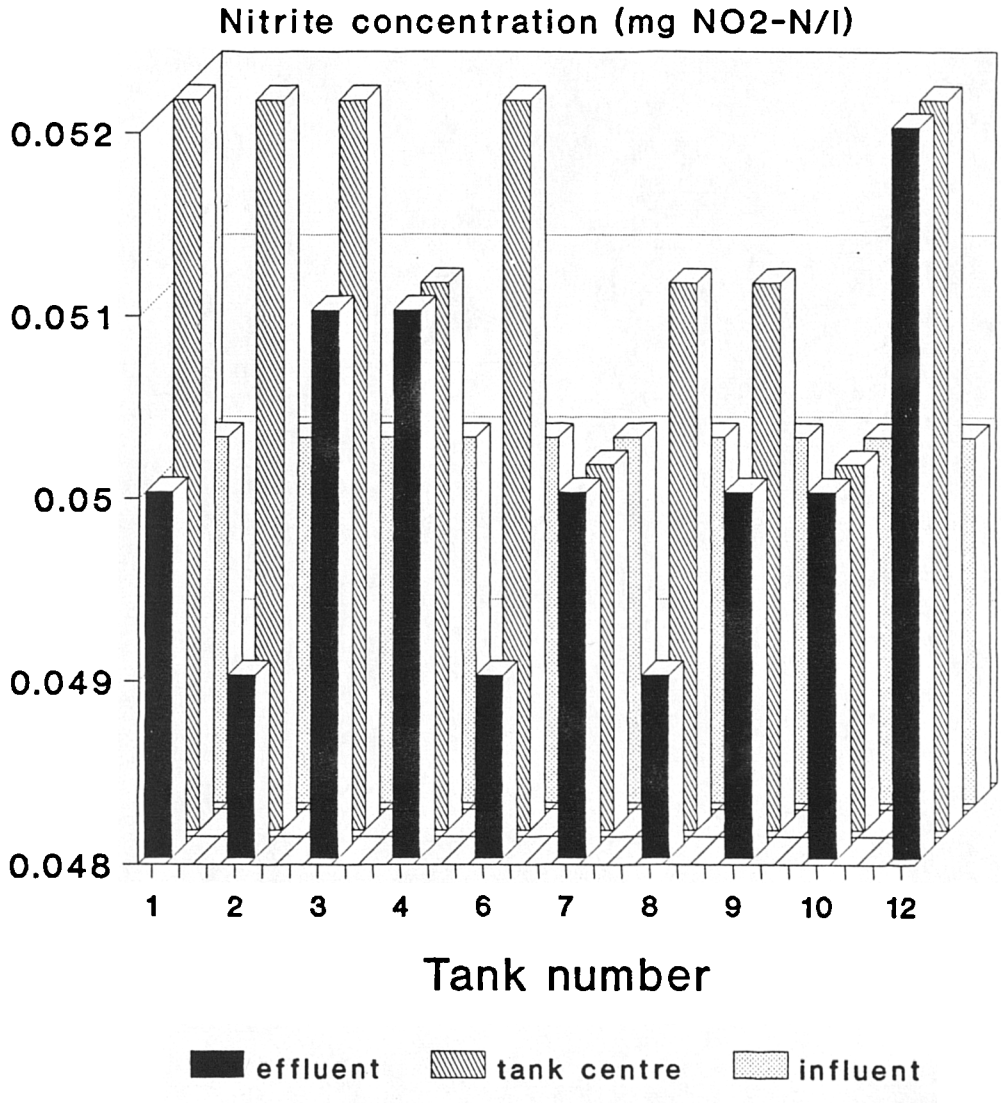


Figure 9.44: Nitrite changes across the treatment tanks



6. Weight: fish in tank 7 (3 cm depth) increased in weight significantly faster than fish in tanks 3, 4, 6, 8, 9, 10 and 12.
7. Weight: fish in tank 4 (12 cm depth) increased in weight significantly faster than fish in tank 8 (6 cm depth).
8. Length: the range of fish lengths during the experiment was 60 - 113 mm at day 0 to 71 - 148 mm at day 56.
9. Length: tank mean fish length distribution was not associated with fish pigmentation.
10. Length: tank mean fish length distribution was not associated with water depth in the treatment tanks.
11. Length: the length of fish in all tanks increased significantly throughout the experiment.
12. Length: the rate of increase in fish length was statistically similar in all tanks.
13. CF: the range of fish CFs was 1.49 - 2.89.
14. CF: the CF of malpigmented fish at day 33 was significantly higher than pigmented fish. This difference was similar to that at the beginning of the experiment and was not evident during other measurement periods.
15. CF: tank mean fish CF distribution was not associated with water depth in the treatment tanks.
16. CF: the distribution of individual fish CFs did not change significantly throughout the experiment.
17. G_W: tank mean G_Ws of fish varied between 0.71 - 1.66 % day^{-1}.

18. G_W: G_Ws of pigmented and malpigmented fish were not significantly different.

19. G_W: treatment tank water depth, between 3 - 18 cm, did not significantly influence the tank mean G_W of fish.

20. G_W: the mean G_W of fish increased significantly, by approximately 0.5 % day^{-1}, with increased treatment tank water depth between 6 - 18 cm, during days 0-13 ($r=0.923$, $df=6$, $p<0.01$).

21. G_W: no clear changes in tank mean G_W with time were apparent.

22. Food consumption: mean food consumption ranged between 0.09 - 3.26 % of body weight day^{-1} (wet/live)

23. Food consumption: no clear associations between the percentage of body weight consumed and either treatment tank water depth or time were evident.

24. FCR: wet feed consumption:live weight gain varied within the range 1.18:1 - 2.56:1. Dry feed consumption:live weight gain varied within the range 0.69:1 - 1.50:1

25. FCR: decreased significantly with increased water depth during the period 33 - 45 days, ie. less food was required per unit fish weight increase in deeper tanks.

26. System water quality:

    temperature = 14.8 - 17.4 ^{o}C

    pH = 7.27 - 7.28

    salinity = 34.5 - 35.4 ppt

DO = 7.1 - 8.1 mg l^{-1} ; 90.2 - 99.5 % saturation

27. 24 h temperature: range during days 52 - 53 was 16.0 - 16.6 ^{o}C.

28. 24 h temperature: was not significantly influenced by water depth.

29. 24 h pH: decreased slowly throughout the sample period and more quickly shortly after feeding.

30. 24 h pH: an increase in the pH drop between the influent and the treatment tank was significantly associated with an increase in water depth in treatment tanks with the same flow rate and in all treatment tanks, but not in tanks with the same residence time.

31. 24 h DO: range = 6.3 - 7.7 mg l^{-1}, 79 - 96.5 % saturation.

32. 24 h DO: no clear fluctuations in DO saturation with respect to the time of day or feeding were evident.

33. 24 h DO: the decrease in DO between the head tank and the treatment tank centres increased significantly with increased water depth in tanks with the same flow rate, in all tanks tested, but not in tanks with the same residence time.

34. 24 h total ammonia: range = 0.019 - 0.133 mg NH_4-N l^{-1}.

35. 24 h total ammonia: a general pattern of approximately constant overnight concentrations followed by peaks 2.5 h after feeding was evident.

36. 24 h total ammonia: concentration increases between the influent and treatment tanks was significantly greater in the deeper tanks.
37. 24 h unionized ammonia: 0.234 - 1.463 μg NH_3-N l^{-1}.
38. 24 h unionized ammonia: increase between the influent and the treatment tanks centres appeared to increase with treatment tank water depth, though this association was not significant.
39. 24 h nitrite: range = 0.034 - 0.064 mg NO_2-N l^{-1}.
40. Within-tank temperature: was 15 ^{o}C in all samples.
41. Within-tank salinity: 34.7 ppt in all samples.
42. Within-tank pH: the mean pH of all tank effluents was significantly lower than the mean pH of all tank centres, which was also significantly lower than the tank influent mean pH.
43. Within-tank pH: mean pH drop between the influents and the tank centres and the influents and the tank effluents increased significantly with increased treatment tank water depth in the tanks with the same flow rate.
44. Within-tank DO: the DO saturation of the influent (head tank) was significantly greater than both tank centre and tank effluent mean DO saturations.
45. Within-tank DO: the decrease in DO saturation occurred primarily between the head tank and the treatment tank centres.
46. Within-tank DO: drop between the head tank and the tank centres increased significantly with increased treatment tank water depth.

47. Within-tank NH_4, NH_3 and NO_2: no significant associations between NH_4, NH_3 and NO_2 concentrations and either sample point location or tank water depth were determined.

9.5 DISCUSSION

9.5.1 Survival

The lack of fish deaths indicates that aspects of the culture of the turbot during this experiment, such as water quality, nutrition and handling, did not induce stress levels which were fatal to the fish. Management of these aspects must have been at least adequate to maintain the stock of turbot. Conclusions summarized in section 9.5.13 indicate that water quality conditions in particular were good rather than merely adequate.

9.5.2 Fish weight and length

Fish weights and lengths, as would be expected, followed similar distributions with respect to time and treatments. The method of randomly sampling both pigmented and malpigmented fish resulted in a distribution of weights and lengths of the two types of fish which were not significantly different at the beginning of the experiment. Any influence of pigmentation was negligible compared with other treatments used during the experiment.

All fish used in the experiment were the same age, so fish better able to thrive in culture conditions, would be expected to be larger, as indicated by a greater weight and length at the beginning of the experiment. Such fish would be expected to grow faster and exhibit enhanced CFs

and FCRs during the experiment, though changes in environmental parameters may produce different responses in different fish irrespective of their history. It was ensured that the size of the fish was not biased by the initial construction of the treatment tank populations, and other aspects such as handling, disturbance and feeding were the same in all tanks. Significant differences in biological parameters between treatments were then attributable to some consequence of the treatment.

Though weight changes were investigated using G_W primarily, this parameter was only calculated for groups of fish. Weight and length data were determined for individual fish, so sample size was larger compared with tank mean or pigmentation type mean data. The significant increases in fish length and weight with time were a further indication that conditions during the experiment were adequate, not only to maintain turbot without mortalities, but also to increase body size. Fish in one of the two shallowest tanks (3 cm depth) increased in weight significantly more than fish in all other tanks with a water depth of 6 cm or more, but not more than fish in the other tank with the same depth. The implication, though not conclusive, that growth was greater in the shallowest tanks will be discussed further in the G_W section.

9.5.3 Condition factor

In section 8.5.3 the use of condition factor as a measure of fish fitness, described by Weatherley (1972), Dave et al. (1975) and Knights (1982), was discussed.

The lack of a significant change in individual fish CFs from the start of the experiment was further evidence, with survival and weight change data, that environmental conditions in the recycle system were adequate to culture turbot. Mean CFs quoted by Heap and Thorpe (1987), referring to fish they considered suited to intensive culture, were similar to the majority of CF values obtained in this experiment, so the assumption that fish were initially fit was valid. Tank mean CFs determined in this experiment were generally greater than those achieved during the experiment described in Chapter 8, in which fish fitness was considered to be poor.

CF was calculated for use as a comparison of fitness between different treatments in this study. Malpigmented fish were shown to have a higher CF, ie. were more fit, than pigmented fish from the same tanks during day 33. Whilst this result was significant, little reliance was placed on it, for two reasons. Firstly, during other periods of the experiment, no significant CF differences occurred between fish with different pigmentation types. Secondly, the difference in CF at day 33 may have been an artifact of a similar, though not significant, difference

which was present during the initial stocking of the treatment tanks.

Treatment tank water depth did not influence the CF of the turbot, within the range of depths tested. The maintenance of either a constant residence time or a constant flow rate at different depths also produced no influence of water depth on CF. A more positive conclusion to be drawn from these results is that even when fish were cultured in a water depth as shallow as 3 cm, with the resulting differences in hydrodynamics and water quality, which are discussed in other sections, the fitness of the fish was unaffected. Consequences of this conclusion will be discussed in Chapters 10 and 11 in relation to other measured parameters.

9.5.4 Specific growth rate

G_Ws reported for turbot have been reviewed in section 8.5.4. It was concluded that a G_W in excess of 1.7 % day^{-1} may be regarded as adequate, though care should be taken when comparing data from different studies with different environmental conditions, particularly temperature. None of the tank mean G_W values determined during this experiment were greater than 1.7 % day^{-1}. The range of G_Ws in this study were generally greater than values quoted by Bowers and Landless (1969) using a restricted diet, Adron et al. (1976 and 1978) Bromley (1980b) and Calcedo Juanes (1988), in which conditions

were considered sub-optimal. G_Ws in this experiment were more similar to those described by Bowers and Landless (1969), feeding to satiation, Purdom et al. (1972) and Lake (pers. comm.). From these comparisons it can be seen that overall the G_Ws achieved in this study were not low enough to cast doubt on some aspect of the culture conditions or treatment, but could not be considered as good. G_Ws during this experiment were a mean of 0.46 % day^{-1} greater than those described in the previous chapter.

Like CF, G_W was calculated primarily to compare the performance of fish under different experimental regimes in which conditions, other than the study treatment, were controlled, rather than with other studies under different conditions. No difference in G_W between pigmented and malpigmented fish was observed. Any differences may have been masked by G_W differences due to treatment, however despite the control of water depth, stocking density and residence time or flow rate, no marked differences due to pigmentation were evident.

This result does not confirm the suggestion by Heap and Thorpe (1987), that the growth rates of malpigmented turbot were greater than pigmented turbot, but they made no mention of the genetic history of the fish. A good conditioned malpigmented genotypic parent may well have produced more viable than average offspring. In this situation better performing juveniles would be the result

of fitter parents. Malpigmentation as an indication of improved performance would be coincidental. No information was available regarding the genetic history of fish used in the present study so this theory could not be tested, but results of this experiment must cast doubts on the possibility that pigmentation is a general indicator, or cause of, improved performance.

During days 0 - 13 of this experiment a clear linear association between G_W and treatment tank water depth in the range 6 - 18 cm was apparent (Figure 9.15). The regression equation describing the association predicted that an increase in water depth from 6 - 18 cm, whilst maintaining SDv constant, but irrespective of whether residence time or flow rate was maintained constant, would be expected to produce an increase in G_W from 0.99 - 1.47 % day^{-1}. This would be a substantial increase in G_W, of 0.48 % day^{-1}. At first sight this G_W association appears to contradict weight results which indicated a greater increase in weight of fish in the shallowest tanks. These weight results however refer to the whole experimental period while the significant G_W result refers only to days 0 - 13. This suggests that the first growth period may not have been representative of the whole experimental period, during which growth in the shallow tanks started slowly, but increased with time.

The result from this experiment is confirmed by data from the experiment described in the previous chapter.

The similarity between the three regression equations detailed in WDE1 and the equation from this chapter, ($y=0.267+0.036x$, $y=0.779+0.038x$, $y=0.744+0.032x$ and $y=0.756+0.040x$ respectively) all describing the significant association between G_W and treatment tank water depth, is further confirmation of the association. The mean **b** (slope) value of these four equations, a measure of the influence of water depth on G_W, indicates that an increase in water depth from 6 to 18 cm would be expected to increase G_W by 0.44 % day^{-1}.

It is unlikely that depth in itself was responsible for the influence. A consequence of depth, such as water quality, feeding behaviour or the hydrodynamics of the tanks was a more probable cause. Having established a circumstantial link between depth and growth, results to be discussed in following sections will be analysed to establish which parameter or parameters were responsible.

Fish in the 3 cm deep tanks did not conform to this association. The tank mean G_Ws of fish from the two 3 cm deep tanks were the same, indicating that the inconsistent results were unlikely to have been caused by chance variation. It is possible that the relationship between the factor which influenced the variation of G_W coincident with depth in the other tanks, was different at such shallow depths. Alternatively another parameter, not controlled in the experiment, such as behavioural response, governed the association at the shallowest

depths. Both these possibilities will be discussed in the appropriate following sections of this chapter.

The significant relationship between G_w and water depth occurred only during the first growth period. It was stated in section 9.3.3 that the fish were deliberately not acclimated to any treatments used during the experiment, so that the effect of the treatment on the fish would be more evident. Results shown in Figure 9.15 indicate that this aim was probably achieved. It is likely that the fish, which were acclimated to the recycle system but not to the experimental treatments, had recovered from their transport to the site and then were affected by parameters associated with water depth, eg. water quality or hydrodynamics. Upon acclimation, during the remaining period of the experiment, the fish in all treatments exhibited a more constant G_w response to their environment. This supports the suggestion by Smith (1979) that having survived weaning, the viability of juvenile turbot is good.

9.5.5 Food consumption and conversion ratio

It is a widely held belief among culturists that juvenile turbot require a moving food source to elicit the feeding response. Casual observations made during this study confirm this theory. A problem associated with a decreased depth of water, particularly as shallow as 3 cm, is that if sinking feed is offered to the turbot, the time

available for the fish to strike the food before it reaches the tank floor, is greatly reduced. Not all strikes are successful, so the time available to make further strikes on the particle is also reduced. This indicates that food consumption may be related to water depth in a tank, assuming that a similar amount of food was offered to fish in all tanks. Food consumption, measured as a proportion of body weight consumed each day, was however similar in fish kept at all depths. Clearly fish cultured in the shallowest depths of water were still able to consume as much food as fish in water depths which allowed ample time to take a sinking food pellet.

Casual observations of fish in the shallower tanks indicated two behavioural acclimations. Firstly these fish were more alert at feeding time and moved quickly to the tank centre where feed was offered. Food was taken much sooner after it struck the water surface. Food was dropped into all tanks from just above water level, to reduce the momentum of the pellet and so increase the time the food remained in the water column. Such careful feeding may be impractical in a commercial situation. Secondly, contrary to popular opinion, food was regularly taken off the floor of the tank by fish in the shallowest tanks. Movement of pellets on the tank floor, which initiated the feeding response, was caused by either vortices resulting from the tank water inflow momentum, or by fish movements. In shallow tanks, fish movements were obviously confined to near the tank floor, so pellets

normally considered as waste on the tank floor were often resuspended and eaten.

The decrease in FCR, ie. a reduction in the amount of food required to produce fish tissue, with increased tank water depth during days 33 - 45, confirmed G_W conclusions that the performance of the turbot was better in the deeper tanks. More stressed and more active fish, would be expected to eat more food per unit increase in body weight. Fish in deeper tanks were then likely to be less stressed than fish in shallower tanks.

The significant influence of tank water depth on FCR occurred only during one growth period and was not coincident with the increase in G_W with increased depth. This result is therefore not conclusive, although the general nature of the trend does agree with other data presented.

9.5.6 Biological parameter conclusions

1. No influence of pigmentation type on any of the biological parameters monitored was determined.
2. Fish weight increased quicker, G_W increased and FCR decreased, ie. improved, with increased water depth within the limitations stated above.
3. Stocking density had no effect on the influence of depth on G_W.
4. Food consumption was unaffected by shallow depths of

water though the method of feeding was modified.

9.5.7 Temperature

Temperature requirements for turbot have been reviewed in section 8.5.8. The recycle system temperature range of 14.8 - 17.4 ^{o}C was within the majority of temperature ranges considered adequate for the growth of turbot, such as those described by Deniel (1976), Person-Le Ruyet *et al*. (1980), Poxton and Allouse (1982) and Poxton *et al*. (1982). It was also higher than ranges described by Heap and Thorpe (1987) and Smith (1979) who achieved adequate growth and survival for commercial turbot culture.

Recycle system water temperatures measured daily at 11.30 h were used to estimate the general temperature of the system throughout the experimental period. Results presented in this study have indicated that temperature fluctuates diurnally. A single daily measurement may not represent the actual temperatures encountered by the fish, so a 24 h water quality survey was carried out. During this survey a diurnal fluctuation of only approximately 0.5 ^{o}C was evident. Addition of this diurnal variation to the experimental period range produced overall expected maximum and minimum temperatures of 14.3 - 17.9 ^{o}C. These extremes in temperature would still not have adversely affected the performance of the turbot.

A weekly temperature periodicity, resulting from the absence of building heating during weekends, was described in Chapter 8. The same situation occurred during the current experiment, but all fish experienced the same conditions and so this should not have biased the results. Differences in water temperature between the treatment tanks were not expected and did not occur, because the difference between air and water temperatures and between treatment tank residence times was small. The improvements in fish performance with increased water depth, summarized in section 9.5.8, were not therefore due to temperature differences.

9.5.8 pH

Section 8.5.9 indicated that a broad range of pH can be tolerated by fish, with mortalities occurring outside the range 5.0 - 9.5, but with a narrower recommended range of 6.5 - 8.5 (Poxton and Allouse, 1982). pH fluctuations during this experiment were well within this recommended range, so pH or the secondary effects of pH on the toxicity of other substances, eg. unionized ammonia, did not adversely affect the fish in the recycle system as a whole. This is assuming that toxic substances, influenced by pH, were not near maximum acceptable limits irrespective of pH, and does not consider local differences in pH due to treatment.

Diurnal fluctuation in pH followed the same pattern

of changes described in section 8.5.9. The resultant fluctuations appeared to be due to two factors: a gradual decrease in recycle system pH throughout the experimental period, probably due to nitrification processes of the filter bacteria; a rapid decrease in pH shortly after feeding followed by some restoration of pre-feeding pH values because of the buffering capacity of the recycle system water. A similar situation appears to have been shown by Honer et al. (1987a), but the buffering capacity of water in the current study was insufficient to produce the diurnal cyclical fluctuations described by Rosenthal et al. (1981). Figures 9.27 and 9.28 show that diurnal fluctuations, though variable in extent, were small, so the pH range measured at 11.30 h daily was a reasonable estimate of pH within the recycle systems. Extremes in pH, hazardous to the turbot, did not occur during the experiment.

The decline in pH between the influent and the treatment tanks was shown to increase significantly with increased water depth. At various periods during the experiment, weight increase, G_W and FCR have been shown to have been significantly influenced by water depth. This is circumstantial evidence indicating that the pH drop was the secondary effect of water depth which influenced these biological parameters. Two aspects of the pH decline however suggest that pH and the biological parameters may vary concomitantly rather than pH influencing these parameters. The maximum pH drop between the influent and

treatment tanks was only 0.05: a very small, and in itself non-significant drop, compared with daily fluctuations of approximately 0.30. Also it would be expected that a smaller pH drop would produce beneficial fish performance values, however the reverse was the case. It is therefore concluded that fish weight increase, G_W, FCR and pH drop were influenced by water depth, but it was unlikely that pH influenced these biological parameters.

SDs in terms of water volume were established during this experiment, so the deeper tanks contained more fish. While the residence time or the flow rate into each tank were maintained the same at all water depths tested, the greater pH decline in deeper tanks indicated that maintenance of these factors was insufficient to compensate for the pH drop caused by the increased quantity of feed consumed by the fish in the deeper tanks. An increased pH with depth was not found in the previous experiment in which the same number of fish were placed in each tank. The number of fish present in the tank and therefore quantity of food consumed, appeared to be the reason for the change in pH drop with depth, rather than hydrodynamic aspects of depth.

9.5.9 Salinity

Salinity of the recycle system water was maintained at approximately 35 ppt +/- 0.5 ppt, which is the average ocean salinity (Pickard and Emery, 1982). The recycle

system water was obtained from the sea and had not been previously used to culture animals. Constituents of the system water were therefore unlikely to differ from seawater and depletion of elements was also unlikely. No rapid changes in salinity which would have produced osmotic shock in the fish and no differences in salinity due to treatment were detected.

9.5.10 Dissolved oxygen

DO levels tolerated by fish were discussed in section 8.5.11. Of particular relevance to this study was the suggestion by Davis (1975) that a DO saturation of greater than 82 % at 15 ^{o}C is required by marine nonanadromous fish to avoid stress symptoms. DO levels measured daily at 11.30 h were well above this recommended level, but DO was also found, in the previous experiment and by Rosenthal et al. (1981; 1982; 1985) and Honer et al. (1987a) to fluctuate diurnally. Whilst DO saturation in the deepest tanks did approach this minimum recommended limit, on only two occasions throughout the 24 h period in one of the tanks did DO fall below the limit, and then only by 3 % saturation. In this tank low DO levels were likely to have adversely affected the fish. Fish in other tanks, in which the water did not fall below 82 % saturation, were unlikely to have been stressed by low DO levels.

Contrary to results presented in Chapter 8 and by

Rosenthal et al. (1981; 1982; 1985) and Honer et al. (1987a), no pronounced cyclical fluctuations in DO occurred diurnally. In some of the tanks in the current experiment, feeding and fish biomass were greater than in tanks described in Chapter 8, so these are not the reasons for the unchanging DO results. Water aeration methods, flow rates and residence times were also the same in the two experiments. The reason for the lack of cycling DO levels in this experiment was therefore unclear.

Water depth was found to be associated with DO levels in the treatment tanks. The drop in DO saturation between the head tank and the treatment tank centres increased with increased water depth, so DO levels in the shallower tanks were preferable, though fish weight increase, G_W and FCR were occasionally better in the deeper tanks. pH results described above, were similar, so this was also evidence which indicated that water quality, in this case DO drop, was associated with water depth, but was unlikely to have influenced the biological parameters directly.

The increased air/water interface surface area to volume ratio in the shallower tanks was probably the explanation for improved DO levels in the shallower tanks. Hydrodynamic mixing was also greater in the shallower tanks, so a greater proportion of the water was contacting the water surface, the primary region for gaseous interchange. The fish biomass was greater in the deeper tanks and therefore oxygen required for respiration and

for BOD, resulting from the larger quantity of food fed, was greater. SDv was however approximately equal in all tanks.

DO differences between the influent and tank centre water were up to a substantial 8 % saturation (Table 9.16). Improved fish performance would be expected in tanks containing water with a higher DO saturation, as indicated by many workers including Davis (1975) and Chiba (1983), but the reverse situation occurred. DO and the biological parameters may then also have varied concomitantly, rather than the former influencing the latter. Only if DO levels had risen to supersaturation levels would an increased DO saturation be expected to adversely influence fish performance as indicated by Cripps (1983). No incidents of supersaturation were detected.

DO levels in replicate treatments during WDE1 decreased with decreased depth, the opposite association to that shown in this experiment. The only treatment difference between the two experiments was the method of calculating SD. SDs clearly influenced the relationship between depth and DO. It was concluded that SDs were inadequate to deplete DO levels in the deeper, longer residence time tanks, so the greater volume of water in these tanks acted as a DO reservoir. This conclusion is supported by the results of this experiment which indicated that the greater number of fish present in the

deeper tanks during WDE2 compared with WDE1 caused the DO level to be reduced, all other parameters being controlled. Rosenthal et al. (1985) has also shown that DO concentrations decreased with increased stocking density.

Several conclusions can be drawn from comparisons between the two experiments. During WDE1, DO increased with decreased SDv, while in WDE2 DO decreased despite a constant SDv. DO saturation did not vary relative to SDv in the same way in both experiments, so SDv was not the sole cause of the DO variation with depth. The lower SDv during WDE2 in the shallower tanks produced an improvement in DO compared with WDE1, so it is likely that the low DO levels in the shallowest tanks during WDE1 were caused by the higher SDv, as suggested in section 8.5.11. It was also suggested that at low SDs the greater volume of the deeper tanks acted as a reservoir for DO which may not occur at greater SDs. SDv of the deeper tanks in WDE2 was greater than in tanks of the same depths in WDE1, resulting in a decrease in DO saturation in these deeper tanks. The previous suggestion therefore appears to be confirmed. A situation more like that described by Rosenthal et al. (1981) was approached during WDE2.

A further complication is that the reverse situation also occurred: DO appeared to be related to SDa. In deeper tanks containing up to 20 fish, DO was reduced and

in shallower tanks containing as few as 3 fish, DO was higher despite constant SDv, flow rate and residence time. It therefore appears that SD, both SDv and SDa, was primarily responsible for determining DO in the treatment tanks irrespective of differences in hydrodynamics at different depths. The influence of hydrodynamics on DO cannot be totally discounted because during WDE1 SDv differences may have distorted the true effect of hydrodynamics on DO, which was then evident during WDE2 when SDv was constant, assuming SDa was unimportant. Alternatively during WDE2, SDa differences may have distorted the true effect of hydrodynamics on DO, which was then evident during WDE1 when SDa was constant, assuming SDv was unimportant.

It should be remembered that water in the culture tanks was not artificially aerated, because this was not considered necessary in a normal situation and because the effects of aeration would have complicated the flow dynamics.

To summarize: either SD differences determined DO at different water depths, or they merely masked the effect of hydrodynamics on DO at different depths and therefore are both important determinants.

9.5.11 Total ammonia and unionized ammonia

The importance of measuring total ammonia (NH_4^+) and

unionized ammonia (NH_3), toxic effects and maximum recommended limits were discussed in section 8.5.12. The maximum level of NH_3-N determined during this experiment was less than one-fiftieth of the maximum recommended limit suggested by Poxton and Allouse (1982) and Wickins (1981), so the turbot were not stressed by this parameter. With the design and management of the recycle system so successfully maintaining low NH_4-N and NH_3-N concentrations, the influence of differences in these parameters due to treatment would be expected to be small.

The magnitude of diurnal fluctuations in NH_4-N concentrations in the treatment tanks were similar to those described by Rosenthal et al. (1981), Honer et al. (1987a) and Poxton and Allouse (1982; 1987). The pattern of fluctuations was most similar to that described by Rosenthal et al. (1981), who described an increase in NH_4-N concentrations after feeding and a gradual decline overnight. NH_4-N concentrations in the current experiment were also similar to WDE1. A general pattern of approximately constant overnight concentrations followed by peaks 2.5 h after feeding was evident. These fluctuations have been shown to be due to feeding, metabolic rate and swimming activity (Burrows, 1964; Brett and Zala, 1975; Tatrai, 1981; Poxton and Allouse, 1982).

Variation in influent (head tank) water NH_4-N concentration did not follow the same pattern of fluctuations as the treatment tanks, indicating that

effluent NH_4-N concentration from the treatment tanks was diluted in the larger volume of the recycle system and that the biofilter bacteria were reducing the NH_4-N concentration.

Despite differences due to species, culture system design or stocking density, the range of diurnal NH_4-N concentrations were similar to those described by Rosenthal et al. (1981) and Poxton and Allouse (1987). This is probably an indication of the ability of filter bacteria to buffer variations in nitrogenous metabolites.

An increase in tank water depth produced a significant increase in NH_4-N concentration difference between the influent and the treatment tank centres, in tanks with the same flow rate or the same residence time. The cause of this increase may have been due to either the poorer mixing which has been shown to occur in the deeper tanks (Chapter 6), or the larger biomass they contained, to maintain a constant SDv in all tanks, which has been demonstrated by Poxton and Allouse, (1987) and Honer et al. (1987a).

The larger biomass of fish in the deeper tanks were consuming more food and therefore as a consequence of metabolism, were excreting more nitrogenous metabolites into the tank. The poorer mixing in the deeper tanks, as indicated by large dead volumes and reduced tc/ti ratios, resulted in a slow replacement of water containing these

metabolites by better quality influent water. Adjustment of flow rate to maintain the same residence time as in the shallower tanks, or adjustment of residence time to maintain the flow rate as in other tanks, did not improve the situation.

High NH_3-N concentrations, indicated by maximum recommended limits, are detrimental to the health and performance of fish. It seems unlikely then that an increase in NH_4-N concentration, which includes NH_3-N, would improve fish performance, even allowing for the possibility of some threshold NH_4-N or NH_3-N concentration between acceptability and toxicity. It is likely then that NH_4-N concentration varied concomitantly with depth as a result of biomass and hydrodynamic factors, which also varied with depth. NH_4-N and NH_3-N concentrations were not the cause of the variation in fish performance with depth.

9.5.12 Nitrite

The origin, toxicity and maximum recommended limits were discussed in section 8.5.13. The maximum NO_2-N concentration determined during this experiment was one fifteenth of that recommended by Wickins (1981) and one twenty thousandth of the 24 h LC_{50} described by Brownell (1980a) for marine fish larvae. NO_2-N concentrations were not therefore large enough to stress the fish and the biofilter can be considered to have functioned well. No

influence of treatment on, or patterns of fluctuation in, NO_2-N concentrations were determined. The site of production and breakdown of nitrite was mainly the biofilter. This device was situated on a different water circuit from the sump tank to that of the treatment tanks. Water from the biofilter, relatively high in nitrite, compared with the system as a whole, mixed with the sump water before reaching any treatment tanks, hence NO_2-N concentration was similar throughout the treatment tank circuit.

No influence of NO_2-N concentration on fish performance was noted or could have been expected. Maximum NO_2-N concentrations were considerably below those expected to stress the fish and differences in NO_2-N concentrations between treatments were even smaller and so unlikely to produce differential fish performance.

9.5.13 Water quality parameter conclusions

1. Temperatures of 14.8 - 17.4 °C or daily variations of 0.5 °C did not stress the fish.
2. pH varied within the recommended range of 6.5 - 8.5 and so did not stress the fish.
3. pH decreased rapidly after feeding each day and slowly throughout the experimental period.
4. The pH difference between the influent and treatment tank centres, which increased with increased water depth, was unlikely to have caused the improvements

in fish performance with increased depth.

5. The pH difference between the influent and treatment tank centres, increased with increased water depth because of the increased biomass present in the deeper tanks.
6. Salinity was maintained within 0.5 ppt of 35 ppt and so did not stress the fish.
7. In only one tank for a period of 2 h daily did DO concentration drop below the minimum recommended 82 % saturation limit, so with this exception DO concentration did not stress the fish.
8. The decline in DO between the influent and treatment tank centres, which increased with increased water depth, was unlikely to have caused the improvements in fish performance with increased depth.
9. Either SD differences determined DO at different water depths, or they merely masked the effect of hydrodynamics on DO at different tank water depths and therefore are both important determinants.
10. NH_3-N concentrations were less than one-fiftieth of the maximum recommended limit so the turbot were not stressed by this parameter.
11. Diurnal fluctuations in NH_4-N concentrations in the treatment tanks, related to feeding, were distinguished.
12. NH_4-N concentrations in the treatment tanks increased with increased water depth because of either poorer mixing in the deeper tanks, or the larger biomass.
13. The increase in NH_4-N concentration difference which

increased between the influent and treatment tank centres with increased water depth, was unlikely to have caused the improvements in fish performance with increased water depth.

14. NO_2-N concentrations detected during this experiment did not stress the fish.
15. pH, DO and NH_4-N conditions were better in the shallower tanks.

9.5.14 Behaviour

The results of casual observations of fish behaviour in different depths of water were the same as those discussed in section 8.5.15. Fish even in the shallowest 3 cm deep tanks were capable of feeding with only minor variations in feeding behaviour, compared with fish in the deeper tanks.

Swimming activity has been shown to increase with increased flow rate and increased local flow velocity on the floor of the tank (section 7.6). Such increases in swimming activity, along with metabolic rate and feeding, have been shown by Burrows (1964), Brett and Zala (1975) and Tatrai (1981) to increase fish excretory products. In tanks with a greater flow rate, such as deeper tanks in which the residence time has been maintained constant, increased swimming rate and therefore worsened water quality and fish performance results would be expected. Burley and Klapsis (1985) however stated that the momentum

of influent water is dissipated to a greater extent in a greater volume of water. While flow rate in five of the ten tanks was increased, to maintain a constant residence time, with increased depth, the local flow velocities at the tank floor would not be expected to increase by the same degree as the influent flow rate, and may even decrease. For this reason, flow rate or local flow velocities cannot be considered responsible for the differences in water quality with water depth. The effect of local flow velocities on the biological parameters cannot be ruled out at present, but will be discussed further in Chapter 11.

9.5.15 Hydrodynamic parameters conclusions

Section 5.7 summarises the conclusions from experiments designed to define the mixing characteristics of tanks managed in the same way as during the experiment described in the present chapter. To aid comparison with biological and water quality data section 5.7 will be summarised here.

1. In tanks with the same flow rate, mixing improved significantly with a decreased depth of water within the range 9 - 18 cm and an associated decrease in residence time.
2. A depth of water between 2.9 cm and approximately 9 cm was required to minimise or eradicate dead volume in the treatment tanks at 2 l min^{-1} flow rate. At greater depths, dead volumes would be expected and at

shallower depths no further decrease in dead volume would be achieved.

3. Mixing was not influenced by depth changes, within the range 6 - 18 cm, with constant residence time and variable flow rates.
4. Adjustment of residence time probably influenced mixing more than flow rate adjustments, within the depth range 9 - 18 cm.

9.5.16 Combined biological, water quality and hydrodynamics discussion

Influences on water quality

Results from the previous experiment indicated that in the tanks which contained fish stocked at the same SDa, hydrodynamic mixing increased with decreased depth, yet water quality declined. There were two possible explanations for the decline in water quality. Firstly, hydrodynamics may have been primarily responsible, because dead volumes were acting as a reservoir of good quality water when SDs were low. Secondly, SDv was greater in the shallower tanks, though biomass was the same, so the effect of the fish on water quality may have been more concentrated.

Partly to determine which of these explanations was correct, the experiment was replicated, with the maintenance of a constant SDv rather than SDa. The

results of this experiment were more complicated and less conclusive than expected. Water quality in the shallow tanks was shown to be better than in deeper tanks. This suggested that hydrodynamic mixing was an important parameter determining water quality, but that during the previous experiment the greater SDv in the shallow tanks biased this result. It has been stated in section 9.5.11, that the reverse situation may be equally possible, ie. that though hydrodynamic mixing was important in determining water quality, the greater SDa in the deeper tanks may have biased the result in the second experiment.

Support for the conclusions from the two water depth experiments, that water quality was influenced by hydrodynamic mixing, comes from suggestions made by: Burrows and Chenoweth (1955; 1970) who were the first to compare hydrodynamics and water quality in different tanks; Burley and Klapsis (1985) who found that certain flow patterns improved the distribution of DO concentrations; and Rosenthal et al. (1982; 1985) who indicated that regions of poor water mixing in the centre of circular tanks contained poor quality water.

The problem with studying the influence of SD on water quality at different depths is that either SDa or SDv must be varied. If, as appears to be the case, variation in either parameter greatly influences water quality despite care to maintain either residence time or flow rate constant, a categorical link between

hydrodynamics and water quality in different depth tanks in which fish are cultured will be difficult to prove.

It seems likely then that hydrodynamics did influence water quality, but that water quality was also dependant on the method of SD. Consequences of this conclusion for a practical aquaculture situation will be discussed in Chapter 11.

Influences on fish performance

There is no doubt that fish performance is influenced by water quality, as reviews by EIFAC (1969; 1970; 1984), Wickins (1981), Poxton and Allouse (1982), Poxton (in press) and many others confirm. What is less certain is that small scale changes in water quality resulting from different hydrodynamic management techniques, eg. water depth changes, affect fish performance significantly. Burrows and Chenoweth (1955; 1970) suggested a correlation between the prevalence of fish disease and flow distributions within tanks, but produced little quantitative evidence of the relationship. Rosenthal et al. (1982) indicated a circumstantial link between tank management, which influenced hydrodynamics, and fish growth and health.

An aim of the current study was to determine the extent of hydrodynamic changes which could be affected before fish performance was influenced. Results from WDE1

must be considered with caution because the growth and fitness of all fish in the study was suspect. Results from WDE2 do give a clearer picture of variations in fish performance with hydrodynamic and water quality changes. Generally fish performance, measured as weight increases, G_W and FCR, improved with increased water depth, worsening water quality and poorer hydrodynamics. Conclusions in previous sections suggest that the changes in water quality with treatment were so small, compared with levels which would be expected to stress the fish, that no influence of water quality on fish performance occurred.

Part of a more positive conclusion, stated in section 8.5.17 was confirmed by results of this experiment. Despite changes in water depth between 3 - 18 cm in the small tanks in which large differences in hydrodynamics resulted, associated changes in water quality were small, provided that water quality levels were well below toxic.

If it is assumed that hydrodynamics and water quality did not affect fish performance during this study, some aspect of turbot behaviour may have been responsible. Differences in turbot behaviour with water depth are most likely to be related to either stocking density, feeding or local flow velocities. Observations on feeding behaviour (section 9.5.14) and feed consumption results (section 9.4.6) indicate that feeding was unaffected by experimental treatment. Results shown in Chapter 7 indicated that swimming activity increased with increased

flow velocity. In shallow tanks with a smaller volume of water to dissipate the influent flow momentum and in which mixing has been shown to increase (section 9.15), flow velocities at the floor of the shallow tanks, would be expected to be greater than in deeper tanks with the same inflow rate. Fish would then be expending more energy swimming in the shallow tanks. Such locomotory energy could otherwise have been used for growth and metabolic processes.

9.6 CONCLUSIONS

1. Generally, hydrodynamics became poorer with increased depth.
2. Generally, water quality declined with increased depth.
3. Hydrodynamics probably influenced water quality, but water quality was also dependant on SD.
4. Aspects of fish performance improved with increased depth at a constant SDv.
5. Changes in water quality with treatment were so small, compared with levels which would be expected to stress the fish, that no influence of water quality on fish performance was reliably demonstrated.
6. Greater flow velocities which occurred at the floor of shallower tanks, increased swimming activity and so may have reduced fish performance in the shallower tanks.

CHAPTER 10

TANK MANAGEMENT MODEL

10.1 INTRODUCTION

Data gathered during the preceding chapters was used to confirm or reject correlations between different parameters associated with the design and management of tanks for culturing turbot. Such descriptive statistics were used to determine influences between variables which occurred in each separate experiment. In addition to these analyses, the experiments were also designed so that results from the different studies could be combined to produce a potentially more powerful analysis of the inter-relationships between different parameters and to generate predictive statistics which model the effect of various changes in the system on other parameters. Such modeling has been suggested by Weatherley (1983) and Rosenthal et al. (1985), but has rarely been conducted in aquaculture situations.

Care should be taken in the interpretation of information gained from such a model because, though the data were obtained from several experiments, results were specific to the conditions encountered during this study. The development of a model for a particular system and set of treatments does however allow the quantification of the inter-relationships between parameters. An insight will be gained into the basic principles operating in

aquaculture systems in general and to specific questions of quantification related to the particular system used in this study.

10.2 METHODS

10.2.1 Model parameters

Summary quantifications of the parameters studied in Chapters 5 - 9 were calculated for use in the construction of a multivariate model of the management treatments and techniques employed. The summarizing statistics were based on the two growth experiments (Chapters 8 and 9), because it was in these studies that the biological parameters, which were used to determine the success of the tank management, were quantified. Water quality parameters were also quantified in these two chapters. The hydrodynamic parameters pertaining to the treatments used throughout this study were examined in Chapters 5 - 7, though only the results of Chapter 5 were used in this model because the other techniques did not yield either strictly comparable or quantifiable data.

The influence of thirteen predictor variables on the following three biological parameters was investigated:

Y_1 = Gw: (% day^{-1}) was calculated as the tank mean overall specific growth rate during the experimental periods.

Y_2 = CF: was calculated as the tank mean of the growth period CF values during the experiments.

Y_3 = FCR: (dry feed weight consumed/ live fish weight increase) was calculated as the tank mean of the growth period food conversion ratios.

These three parameters were chosen to indicate the influence of the predictor parameters because the results of previous experiments indicated that they were influenced by treatment, or because they were shown to be the main indicators of fish stress, performance and fitness which will be influenced by environmental parameters. The thirteen predictor variables were defined as follows:

X_1 = Biomass: (g) the experimental period mean total weight of turbot in each tank.

X_2 = SDv: (g m^{-3}) the experimental period mean stocking density of turbot in each tank expressed as a factor of tank water volume.

X_3 = SDa: (g m^{-2}) the experimental period mean stocking density of turbot in each tank expressed as a factor of tank floor surface area.

X_4 = Depth: (cm) water depth.

X_5 = Volume: (l) tank water volume.

X_6 = First response time: (min) time taken for a dye tracer, injected as a pulse input at the tank inlet, to be detected in the effluent stream.

X_7 = t_c/t_i: ratio of the actual residence time to the calculated ideal mean residence time.

X_8 = %Vd: (%) the percentage of the tank water volume which was calculated to be dead volume.

X_9 = Flow rate: (l min^{-1}) rate of tank influent water flow.

X_{10}= Residence time: (min) calculated ideal mean residence time (t_i).

X_{11}= Unionized ammonia: (μg l^{-1}) tank mean of the unionized ammonia concentration differences between the influent water and the treatment tank water during a 24 h period.

X_{12}= Dissolved oxygen: (% saturation) tank mean of the dissolved oxygen percentage saturation differences between the influent water and the treatment tank water during a 24 h period.

X_{13}= pH: tank mean of the pH differences between the influent water and the treatment tank water during a 24 h period.

Some of the methods employed to quantify the parameters listed above, such as the measures of stocking density, were necessarily generalizations because variations in the parameter values were large during the period of the experiments. Temperature and salinity, though expected to influence fish fitness and performance (see sections 8.5.8 and 8.5.10 respectively), were not included in this model because variation in these

parameters was small, so their change in influence on other parameters would not be great.

10.2.2 Statistical analysis

A step-wise multivariable regression analysis was calculated using the Minitab statistics package (Ryan et al., 1985). The statistical requirements of the distribution of the data, stated by Ryan et al. (1985), were approximately fulfilled, though the distribution of the biological data did not appear normal. Sample size was too small to formally test this assumption statistically, but previous analyses of normality on the raw data indicated that the results of the parameters were approximately normally distributed.

The number of parameters in relation to the sample size was too large to allow a single equation to summarize the situation. Also several of the parameters varied directly as a factor of others, eg. depth and volume, and so results of analyses would have indicated an unrepresentatively high correlation coefficient. The data was therefore separated to produce several regression equations. Data was also separated into five groups of parameters: biological, Y_1 - Y_3; stocking, X_1 - X_3; hydrodynamic, X_4 - X_8; flow X_9 - X_{10}; and water quality X_{11} - X_{13}. Equations were derived to examine the influences of one group of parameters on parameters from other groups. The influence of water quality on

biological parameters, stocking on biological parameters, hydrodynamics on biological parameters, hydrodynamics on water quality and stocking on water quality, was examined.

The significance level of the **b** value of each **x** parameter in each equation was calculated to indicate the probability that the value was different from zero and that it was therefore statistically significant in explaining the influence of each **x** variable on the **y** parameter. Also the significance of the whole equation was similarly calculated. A value for r^2, the square of the correlation coefficient, was calculated to indicate the proportion of the total variation of **y** about its mean which was explained by the equation.

10.3 RESULTS

The data used in the multivariable regression analyses are shown in Tables 10.1 and 10.2. Many analyses were calculated, but only those which produced significant results were listed in Table 10.3.

Equations 1 - 3 indicate that water quality, stocking density and biomass significantly influenced G_W. In particular NH_3, DO, pH, SDa and biomass significantly influenced G_W. No other parameters were significantly associated with G_W. No parameters were associated with CF. FCR was influenced by two of the water quality parameters, DO and pH (equations 4 and 5), though only the

Table 10.1: Data used in model determination - water depth experiment 1

tank no	Y_1 G_w	Y_2 CF	Y_3 FCR	X_1 bio-mass	X_2 SDv	X_3 SDa	X_4 depth	X_5 vol	X_6 1st resp	X_7 tc/ ti	X_8 %Vd	X_9 flow rate	X_{10} res t	X_{11} Un amm	X_{12} DO	X_{13} pH
1	0.79	1.70	1.49	76.2	12330	379	3	6.18	-	-	-	0.57	10.84	-0.52	4.06	0.014
2	0.86	1.76	1.26	72.7	5882	362	6	12.36	1.17	0.883	11.71	1.14	10.84	-	-	-
3	0.74	1.77	1.29	79.1	4261	394	9	18.54	0.59	0.918	8.25	1.71	10.84	-0.15	1.44	0.001
4	0.78	1.72	1.97	66.1	2674	329	12	24.72	0.57	1.047	-4.73	2.28	10.84	-	-	-
5	0.92	1.75	1.96	71.2	2304	354	15	30.90	0.56	1.142	-14.23	2.85	10.84	-	-	-
6	0.84	1.68	1.29	76.3	2058	380	18	37.08	0.53	0.908	9.22	3.42	10.84	0.26	0.91	0.002
7	0.62	1.70	2.28	63.2	10227	314	3	6.18	-	-	-	2.00	3.09	0.35	1.77	0.002
8	0.67	1.79	1.70	85.1	6885	423	6	12.36	1.05	0.815	18.48	2.00	6.18	-	-	-
9	0.96	1.74	1.27	77.6	4186	386	9	18.54	0.48	0.937	6.29	2.00	9.27	0.08	0.87	0.001
10	1.08	1.74	0.98	78.8	3188	392	12	24.72	0.60	0.866	13.41	2.00	12.36	-	-	-
11	0.67	1.72	-	70.7	2288	352	15	30.90	0.65	0.821	17.85	2.00	15.45	-	-	-
12	1.06	1.72	1.13	77.3	2079	385	18	37.08	0.61	0.821	17.88	2.00	18.54	0.22	1.25	0.001

See key for heading definitions:-

Table 10.2: Data used in model determination - water depth experiment 2

tank no	Y_1 G_w	Y_2 CF	Y_3 FCR	X_1 bio-mass	X_2 SDv	X_3 SDa	X_4 depth	X_5 vol	X_6 1st resp	X_7 tc/ ti	X_8 %Vd	X_9 flow rate	X_{10} res t	X_{11} Un amm	X_{12} DO	X_{13} pH
1	1.33	1.80	0.98	51.2	8285	255	3	6.18	-	-	-	0.57	10.84	-0.19	2.73	0.018
2	1.11	1.87	0.98	149.2	12071	742	6	12.36	1.17	0.883	11.71	1.14	10.84	-	-	-
3	1.34	1.80	0.97	169.9	9164	845	9	18.54	0.59	0.918	8.25	1.71	10.84	-0.26	2.51	0.021
4	1.34	1.82	0.80	256.6	10380	1277	12	24.72	0.57	1.047	-4.73	2.28	10.84	-	-	-
6	1.50	1.85	0.82	364.7	9835	1814	18	37.08	0.53	0.908	9.22	3.42	10.84	-0.21	6.36	0.037
7	1.30	2.00	0.84	84.5	13673	420	3	6.18	-	-	-	2.00	3.09	-0.18	0.76	0.004
8	1.12	1.85	0.97	124.0	10032	617	6	12.36	1.05	0.815	18.48	2.00	6.18	-	-	-
9	1.25	1.82	0.95	174.3	9401	867	9	18.54	0.48	0.937	6.29	2.00	9.27	-0.23	3.48	0.026
10	1.36	1.83	0.88	213.4	8633	1062	12	24.72	0.60	0.866	13.41	2.00	12.36	-	-	-
12	1.40	1.87	0.77	370.3	9987	1842	18	37.08	0.61	0.821	17.88	2.00	18.54	-0.39	8.03	0.053

See key for heading definitions:-

Key to Tables 10.1 - 10.3
Data used in model determination

Y_1	=	G_w	= specific growth rate (% day^{-1})
Y_2	=	CF	= condition factor
Y_3	=	FCR	= food conversion ratio (dry feed/live fish weight)
X_1	=	biomass	= total fish weight (g)
X_2	=	SDv	= stocking density by volume (g m^{-3})
X_3	=	SDa	= stocking density by tank floor area (g m^{-2})
X_4	=	depth	= water depth (cm)
X_5	=	volume	= water volume (l)
X_6	=	1st resp	= first response time (min)
X_7	=	t_c/t_i	= ratio of t_c:t_i
X_8	=	%Vd	= percentage of tank water volume which was dead space
X_9	=	flow	= inflow rate (l min^{-1})
X_{10}	=	res t	= ideal mean residence time t_i (min)
X_{11}	=	un amm	= unionized ammonia concentration difference between the influent and the treatment tank centre: influent - treatment tank (ug NH_3-N l_{-1})
X_{12}	=	DO	= dissolved oxygen concentration difference between the influent and the treatment tank: influent - treatment tank centre (% saturation)
X_{13}	=	pH	= pH difference between the influent and the treatment tank: influent - treatment tank centre

Table 10.3: Regression equations of tank management model

equation	regression equation	R^2
1	$Y_1 = 1.09 - 0.103\ X_{11} - 0.200\ X_{12} + 37.5\ X_{13}$	74 %
2	$Y_1 = 0.699 + 1.5\times10^{-5}\ X_2 + 3.67\times10^{-4}\ X_3$	55 %
3	$Y_1 = 0.776 + 2.09\times10^{-3}\ X_1$	51 %
4	$Y_3 = 1.10 + 0.558\ X_{11} + 0.282\ X_{12} - 44.6\ X_{13}$	60 %
5	$Y_3 = 1.13 + 0.272\ X_{12} - 48.9\ X_{13}$	52 %
6	$Y_3 = 1.66 - 1.8\times10^{-5}\ X_2 - 4.56\times10^{-4}\ X_3$	34 %
7	$Y_3 = 1.56 - 5.15\times10^{-4}\ X_3$	32 %
8	$Y_3 = -0.694 + 2.05\ X_7$	24 %
9	$X_{12} = -0.397 + 1.13\times10^{-4}\ X_2 + 3.4\times10^{-3}\ X_3$	82 %
10	$X_{12} = 0.35 + 0.018\ X_1$	78 %
11	$X_{13} = 9.04\times10^{-3} + 1.0\times10^{-6}\ X_2 + 2.5\times10^{-5}\ X_3$	85 %

Highlighted values indicate statistical significance
Highlighted R^2 values indicate that the whole equation was significant
See key for variable definitions

influence of pH was significant. t_c/t_i and SDa, not SDv, significantly influenced FCR (equations 6 - 8).

DO was found to be dependant on biomass and SDa, but not SDv (equations 9 and 10). pH was highly correlated with SDa (equation 11). No influence of any of the hydrodynamic parameters on water quality, G_w or CF was evident.

10.4 DISCUSSION AND PREDICTIONS

10.4.1 Influences on biological parameters

G_w and FCR were shown by the model (equations 1, 4 and 5 in Table 10.3) to be influenced by aspects of water quality. G_w improved substantially with an increased drop in pH between the influent and the tank water: the reverse correlation to that expected. The improvement in G_w was not a consequence of reductions in unionized ammonia concentrations in the tanks with the decrease in pH, because no significant influence of unionized ammonia, statistically analysed separately, was determined.

Closer examination of the data indicated that the associations between pH and both G_w and FCR, though highly probable, may not have been representative of the situation. G_w during WDE1 was low and varied little between treatments, also five of the six pH differences

were on the limit of resolution. pH was not found to influence G_W during this experiment. In WDE2 G_Ws and pH differences were larger than during WDE1. Overall a bimodal, rather than a normal distribution of G_W with respect to pH differences was apparent. The use of the multiple regression analysis in this situation was not reliable, so it was difficult to distinguish whether G_W varied concomitantly, or was associated with pH difference. The former explanation was more likely because of the distribution of the data and because the correlation was the reverse of that expected.

G_W also improved with a decrease in DO difference. Examination of the data indicated that more reliance could be placed on this result because the range of DO differences was similar in both growth experiments. In lower oxygen situations within the treatment tanks relative to the influent, G_W was reduced.

SDa and biomass significantly influenced both G_W and FCR. The floor surface area of all tanks was the same, so SDa varied as a factor of biomass, hence the similarity of the analysis results. Increased SDa resulted in improved G_W and FCR, again contrary to the expected result. The improvements may have been the result of behavioural responses to isolation in low SD treatments. It was however more likely that a similar deviation from normality in the distribution of the data from the two growth experiments, as described above was responsible for

this association. Again, it was not possible to distinguish whether these two biological parameters, varied with SDa concomitantly, or were associated.

FCR was also significantly influenced by tank mixing (t_c/t_i) and therefore also Vd because these two hydrodynamic parameters varied as a function of each other. r^2 which quantified the extent of the association indicated that the equation (8) only accounted for 24 % of the variation. No significant influence of any parameter on CF was determined. Results shown in Figures 8.5, 8.6, 9.5 and 9.6 indicated that CF varied little throughout the experiment, so fluctuations in CF with treatment were not expected. Fish fitness was therefore unaffected by treatments used in this study.

10.4.2 Influences on water quality parameters

No influence of any hydrodynamic parameters on water quality was determined, despite the large changes in the flow dynamics in the tanks indicated in Table 10.3 and Chapters 5 - 7. An approximately normal distribution of hydrodynamic and water quality data was evident so more reliance was placed on these results.

The DO drop between the influent and the treatment tanks increased significantly with increased SDa and biomass, indicating the greater oxygen requirements of a greater quantity of fish. Data indicated that an increase

in water volume with no increase in the biomass of fish present, so that SDa was constant, resulted in no significant improvements in DO levels. Similarly, the depletion of oxygen resulting from an increased biomass in a tank, was not fully offset by a proportional increase in the water depth. Using equation 9 (Table 10.3) to illustrate this suggestion:

Starting with a biomass of 50 g in a tank containing 9 cm depth of water:

$$DO = -0.40 + 1.13 \times 10^{-4} \times SD_v + 3.4 \times 10^{-3} \times SD_a$$
$$DO = -0.40 + 1.13 \times 10^{-4} \times 2697 \text{ g m}^{-3} + 3.4 \times 10^{-3} \times 249 \text{ g m}^{-2}$$
$$DO = 0.76 \text{ \% saturation drop}$$

A doubling in the biomass at the same depth of water will result in the following increased DO drop:

$$DO = -0.40 + 1.13 \times 10^{-4} \times 5394 \text{ g m}^{-3} + 3.4 \times 10^{-3} \times 498 \text{ g m}^{-2}$$
$$DO = 1.91 \text{ \% saturation drop}$$

However if tank water depth and therefore water volume was also doubled the DO drop would be decreased, but not to the initial value of 0.76 % saturation:

$$DO = -0.40 + 1.13 \times 10^{-4} \times 2697 \text{ g m}^{-3} + 3.4 \times 10^{-3} \times 498 \text{ g m}^{-2}$$
$$DO = 1.65 \text{ \% saturation drop}$$

This indicates that increases in the biomass in a

tank were not fully compensated for by increased depth and therefore volume, to maintain the same SDv, despite the maintenance of a constant flow rate or residence time.

Poxton and Allouse (1987) showed that ammonia concentration decreased in culture tanks with increased flow rate. A similar situation was expected in this study, because increased flow rate and decreased residence time would be expected to flush through wastes and supply nutrients faster. No such association between flow rate or residence time and water quality, such as that shown in Chapter 8, was determined using this model. This indicates that the range of water quality variables was not great in this study, relative to critical levels, and that associations determined in WDE1 were not the same in WDE2. Such small differences in water quality were unlikely to be important for culture purposes anyway.

The decrease in pH with increased SD confirms suggestions by Wickins (1981) that pH decreases in a culture system due to the accumulation of CO_2. Equation 11 indicates that at a constant SDv, a change in SDa of approximately 3.5 kg m^{-2} would only cause a pH drop between the influent and the treatment tank of pH 0.1 assuming the relationship between the parameters remained constant to these extrapolated SDa values.

10.4.3 General conclusions

If it is assumed that the skew in the biological data did not invalidate aspects of the multivariant analysis which included this data, the model proposed indicates that the hydrodynamics of the tanks had little or no direct influence on fish fitness or performance, or even on water quality. SDa was the main factor which determined DO and pH in the treatment tanks. These two water quality parameters also significantly influenced G_w, as did SDa directly. This suggests that, in this study, differences in the SDa, and biomass, caused water quality changes, which in turn influenced fish performance. It is stressed that this conclusion refers to the particular conditions which were prevalent in this study. In particular it is possible that in more extreme mixing conditions, hydrodynamic parameters may assume a greater importance in relation to SD.

If the use of the multivariant analysis is not applicable to the biological data in this study, the model may be considered to be of little descriptive or predictive value regarding these parameters. SDa was however shown to influence DO and pH, which were not themselves influenced by tank mixing. The range of treatment conditions did not appear extreme enough to produce a wide range of influences on other parameters. Tank mixing conditions were however shown to vary greatly (Chapter 5), but still produced little effect on water

quality or biological parameters. Thus conclusions stated in sections 8.5.17 and 9.5.16, which indicated that it may be possible to alter the management of a tank greatly, causing substantial changes in flow dynamics, with little effect on the fish, were confirmed.

In summary the results indicated that the hydrodynamics of the tanks did not influence water quality, fish fitness or performance. It was more probable that stocking density and biomass were the major influences on water quality and this in turn may have contributed to differences in fish growth and FCR. Differences in fish performance and fitness between the two WDEs limited the application of a model based on multivariate analyses.

10.5 Summary of conclusions

1. Differences, in the biological data, between the two growth experiments cast doubt on the validity of the use of multivariant analyses with these parameters. The use of the analysis with water quality and hydrodynamic data was still justified.
2. Hydrodynamics had little influence on biological or water quality parameters.
3. Increased SDa significantly increased the decline in pH and DO between the influent and treatment tank water. A 249 g m^{-2} increase in SDa was expected on average to decrease DO saturation by 1 % and a

4 kg m^{-2} increase to decrease pH by 0.1.

4. DO may have influenced fish G_w and pH may have influenced fish FCR.

5. The model indicates that SDa influenced water quality and this in turn may have influenced the biological parameters.

CHAPTER 11

OVERALL CONCLUSIONS

11.1 Introduction

The purpose of this chapter is to draw conclusions based on the different aspect of this study, not to repeat the previous detailed discussions. The number of possible inter-relationships was complex and results were often contradictory, so care was taken not to extrapolate too greatly from these data. The results were however adequate to allow all of the project objectives (Chapter 2) to be fulfilled. This chapter will also discuss the success and relevance of the experiments and analyses conducted in this study and suggest further work which may be conducted.

11.2 Hydrodynamics

Three methods were employed to study the hydrodynamics of the treatment tanks: exit age distribution, flow visualization and flow velocity studies. Of these techniques, the former was the most suitable for this type of study because it yielded summarizing statistics which quantified mixing in the tanks. These could then be compared with other parameters such as water quality. The other two techniques were useful for providing a picture of tank mixing but results gained were less comparable with other aspects. All three

methods indicated the same mixing situation in the tanks and so can be considered accurate, but with different levels of precision. Future studies of this type, which compare a wide range of parameters, may be better to concentrate on the exit age distribution technique to estimate the hydrodynamics, unless an overall picture of flows is required. The method developed in this study and described in section 5.4.4 is particularly suitable for aquaculture applications and may be conducted with animals present.

Either of the two mixing conditions described by Dankwerts (1953) and in section 5.3.1: plug flow and perfect mixing, would be suitable in an aquaculture situation. These two conditions are however almost impossible to achieve in practice. A review of the tank design literature (Chapter 3) indicated that in both rectangular raceways and circular tanks the object of the design and management was to achieve as close to a plug flow situation as possible. In studies of raceways, such as that described by Haskell et al. (1960), a plug flow was difficult to achieve. In circular tanks, such as those described by Rosenthal et al. (1982), a non-linear plug flow was almost impossible to achieve, so in practice a perfect mixing situation was attempted. Depth was the primary treatment investigated during the current study. One of the hypotheses tested was that depth could be reduced in tanks for culturing demersal species, without detrimental effects on flow dynamics. Results of

Chapter 6 however indicated that boundary layer drag became increasingly important as water depth was decreased. Such drag will disrupt a plug flow. In shallow tanks of any shape, plug flow will be difficult to achieve. For this reason and because principles determined in this study were to be applied to a range of tank designs, including circular tanks, the perfect mixing situation was considered preferable and easier to approximate. Indeed the boundary layer drag in shallow tanks may be used to increase mixing.

Self-cleaning has not been covered in great detail in this study though it has been shown to be an important aim for optimising flow dynamics. The tanks used in this investigation were not self-cleaning and would have been extremely difficult to make so. No results pertaining to self-cleaning were therefore obtained and conclusions with respect to this criterion were not made.

11.3 WATER QUALITY

Standard water quality techniques were adopted during this study and can be considered adequate for the purpose. Nitrite and total ammonia concentrations were determined manually and so the number of analyses which could be conducted was limited. A greater number of sample points at different locations within a tank, as described by Rosenthal *et al*. (1982), may have been useful in qualifying the relationship between tank hydrodynamics and

water quality. Water quality throughout the study was not critical and varied little, despite large changes in tank hydrodynamics.

11.4 BIOLOGICAL PARAMETERS

Conclusions based on biological parameters quantified in Chapter 8 must be treated with caution, because comparison of these parameters with values achieved during experiments described in Chapter 9 (sections 9.4.4-9.4.7) and published values (sections 8.5.3 - 8.5.7) indicated that the majority of the fish were stressed irrespective of treatment.

11.5 INTER-RELATIONSHIPS BETWEEN PARAMETERS

Throughout this study, tank hydrodynamics had little direct influence on water quality, fish fitness, or performance as discussed in Chapters 8 - 10. Either the data or analyses used in this study were inadequate to determine such associations, no associations existed, or other non-hydrodynamic parameters masked an association. The latter explanation was most likely. Ranges in water depth (3 - 18 cm) produced profound changes in tank mixing (eg. $\%V_d$ range of approximately 1 - 25 %) and wide ranges in the biological parameters were determined (eg. overall mean G_w range of approximately 0.5 - 1.2 % day^{-1}, Figure 8.11). The range in water quality was not great compared

with maximum levels expected to stress fish (see sections 8.5.8 - 8.5.14 and 8.5.17).

Circumstantial links between different parameters were indicated by many workers including Burrows and Chenoweth (1955; 1970), Rosenthal et al. (1982; 1985) and Burley and Klapsis (1985). A definite link was indicated by Poxton and Allouse (1987). It is therefore probable that associations between tank hydrodynamics and other parameters did exist, as discussed in section 9.5.16. The tank management model described in Chapter 10 indicated that water quality and possibly the biological parameters were primarily influenced by SDa, even at the low stocking densities used in this experiment, compared with other research studies and commercial operations (see Chapter 3). This conclusion, drawn from the evidence of different experiments in this study and previously published results, indicates that while different tank hydrodynamics resulting from changes in water depth, residence time and flow rate, probably did influence water quality and fish fitness and performance, the effects of stocking density and biomass were dominant.

11.6 CONSEQUENCES OF ALTERING TANK MANAGEMENT

Several conclusions may be drawn from the former statement, some of which have been indicated previously (sections 8.5.17, 9.5.16 and 10.4.3). Three objectives of this investigation (Chapter 2) were that results, based on

experiments and a review of the literature, could be used to indicate: 1. the influence of tank maintenance techniques on various hydrodynamic, water quality and biological parameters to indicate the most suitable environment specific to this study; 2. to indicate the most suitable design of tank currently available; 3. to indicate a new design specifically for turbot culture. The first aim can be fulfilled, though results from different experiments were not always consistent. The second two aims must be achieved using subjective assessments based on extrapolations of data. Such recommendations must be treated with caution, yet should still be made as an aid to establishing a starting point for future culturists and researchers.

In general terms (for more detailed analyses see the relevant chapters), a reduction in water depth from 18-6 cm caused pronounced changes in tank hydrodynamics, but these changes had less effect on water quality than differences due to stocking density and biomass. At greater stocking densities than those in this study a greater influence of biomass on water quality would be expected. The effect of hydrodynamics in such a situation is less clear. Two conditions are possible: either the influence of hydrodynamics on water quality would be dominated by the effects of stocking density; or, as water quality approached critical levels, it would be more important, than during this study, to ensure that influent water was mixed throughout the tank and that poor quality

water was removed as quickly as possible. In this case, hydrodynamics would assume a greater importance in relation to stocking density. Specific results are not available to answer this question, though the circumstantial links between flow dynamics and water quality, mentioned above, suggest that the latter situation would be more likely.

Within the range of treatments established and equipment used in this study, results indicated that flow rate can be reduced with water depth, so that residence time is maintained constant, and no significantly detrimental effects on fish fitness and performance will result providing that the biomass is reduced as a factor of tank volume. If flow rate is at least maintained constant, water quality will be improved with decreased depth and biomass. Results from Chapter 8 indicating the consequences of maintaining a constant biomass with decreased depth, are not clear because all the fish appeared stressed irrespective of treatment, but water quality and fish performance did decline occasionally. A reduction in water depth could then be a way of improving water quality for no extra water flow requirements, but at the expense of biomass of fish cultured. This was however the most cautious scenario. With only a marginal risk of decreased fish performance, water depth and flow rate could have been decreased, but at the expense of water quality.

The optimum management strategy for this study will depend on various circumstances and priorities. For example if the quantity of available water is limited, water depth may be reduced to allow an decrease in residence time and an increased flushing through of tanks.

Alternatively if G_w and FCR are the overriding considerations, water depth should be increased with an associated increase in flow rate to at least maintain the same residence time. Depth can be decreased, but flow rate must be increased to offset the expected small deterioration of water quality and fish performance. The situation is not therefore simple, and a straightforward decrease in water depth cannot be recommended without the above provisos.

There are several advantages in reducing water depth in addition to better water mixing. The increased mixing should increase self-cleaning by carrying waste out of the tank. A decrease in the quantity of food wasted may result from the increased SDv because food on the floor of the tank is resuspended by the movement of the fish in the more confined volume (see section 8.5.15). Flushing, ie. both calculated and actual residence time, will be increased for a given flow rate as depth is reduced (Chapter 5). Alternatively the reduction in flow for a given residence time will result in savings in pumping costs and water requirements. Tank construction may be less substantial, because the weight of water retained

will be less, with subsequent savings in the cost of tank materials. The reduction in volume, and therefore weight of water per unit floor area, may allow stacking of tanks, such as that suggested by Prevost (1941) (section 3.6) to increase production within an available fish farm floor area.

Disadvantages include a risk of decreased fish performance if biomass is maintained constant and flow rate is not increased or residence time decreased. Water quality will be less stable (section 8.5.14) which may be particularly serious during emergencies such as a failure of the influent water supply.

From this comparison of advantages and disadvantages, it appears that a reduced water depth to a minimum of 6 cm is likely to be more advantageous when culturing juvenile turbot within the size range of fish in this study. One point mentioned above cannot however be answered by results of this study and so would require further work: it is not known whether the same inter-relationships between the parameters apply at high stocking densities in which water quality may approach critical levels.

11.7 TANK DESIGN SUITABILITY

Results of this study indicate that many designs of tank may be suitable for the culture of turbot provided that substrate area is adequate to allow settlement. This

would exclude several designs such as silos, triangular or bell-shaped tanks, without substantial adaptations which may cause poor flow dynamics. Shallow tanks may be employed provided that care is taken to ensure that stocking densities are not too great for the available flow rates. In these shallow tanks, mixing will be greater but care must be taken to ensure that water quality is maintained and local flow velocities are not so great that the fish have to swim to maintain position. Circular tanks, despite often suffering from dead areas, have advantages in terms of water conservation and may be particularly useful in this context. A decrease in water depth, with only a small chance of detrimental effects on fish performance, or an increase in flow rate would be expected to decrease these dead areas. A reduction in the capital cost of manufacturing circular tanks with a reduced depth may be expected. The Burrows pond (Burrows and Chenoweth, 1970) with its baffles and carefully located effluent pipes may be particularly suited to shallow turbot culture.

CHAPTER 12

SUMMARY OF MAIN CONCLUSIONS

Chapter 3

1. Previous workers have indicated that the consequences of tank design and management are far ranging and are often overlooked.
2. Large carefully managed circular tanks appear, from results presented in the literature, to be the most suitable design for the culture of turbot.
3. The Burrows pond, though possibly more expensive to construct than simpler designs, may prove suitable for turbot culture.

Chapter 5

4. If water depth was decreased from 18 - 9 cm, while flow rate was maintained constant (2 l min^{-1}), with associated decreases in residence time (18.54 - 9.27 min), mixing significantly improved (Vd: 6.95-1.37 l)
5. Increased flow rate (0.5 - 13.0 l min^{-1}) into the tanks greatly improved mixing at low flow rates, but increased hydrodynamic benefits declined with increased flow rate greater than that required to eradicate dead volume (approximately 2.5 l min^{-1}).
6. A reduction in water depth was an alternative to an increase in flow rate as a means of achieving

improved, ie. increased mixing, in the tanks.

7. Tank hydrodynamics were unaffected by fish stocking density (0 - 50 fish $tank^{-1}$) during this study.

Chapter 6

8. Flow separation, dead volumes and recycling of water in deeper tanks was shown to occur.
9. Boundary layer frictional drag became an increasingly important factor determining flow dynamics in shallower tanks.

Chapter 7

10. A preference of the fish to settle in regions of the tank with vertical eddies was suggested and may indicate that a "microclimate" at the floor of the tank, in regions with a disrupted boundary layer, but low horizontal flow velocities to maintain position against, was important to the fish.
11. Swimming activity increased with increased flow rate, so more energy was expended swimming in faster flow rate tanks.

Chapter 8

12. The performance of fish stocked at a constant SDa, occasionally improved with increased depth (eg. an increase in water depth from 3 - 18 cm increased G_W

by a mean of 0.48 % day^{-1}), though all fish were shown to be stressed irrespective of treatment.

13. Water quality in tanks stocked at a constant SDa improved marginally with increased water depth and appeared to fluctuate less diurnally.
14. Despite the range of treatments adopted in this experiment and the resulting range of mixing parameters, water quality did not approach critical levels, so little influence of depth on the indicators of fish fitness and performance was determined.

Chapter 9

15. Aspects of fish performance improved at a constant SDv with increased depth (eg. increased water depth from 6 - 18 cm increased G_w by approximately 0.44 % day^{-1}), despite a decline in water quality. Changes in water quality with treatment were so small, compared with levels which would be expected to stress the fish, that no influence of water quality on fish performance was reliably demonstrated.
16. Hydrodynamics probably influenced water quality, but water quality was also influenced by SD.

Chapter 10

17. When data from the different experiments were combined to produce a model of the culture situation,

it was determined that hydrodynamics did not influence the biological or water quality parameters, despite the large range of water depths and hydrodynamics relative to the size of the tanks.

18. It was more probable that SD and biomass were the major influences on water quality and this in turn may have influenced fish performance.

Chapter 11

19. The optimum strategy for the management of a tank will, based on results presented in this study, depend on various circumstances and priorities, such as the availability of water, minimum biomass requirements and preferred water quality.

20. The advantages of reducing water depth to as little as 6 cm in a culture tank containing juvenile turbot are more numerous than the disadvantages. Conditions must be carefully monitored if such a strategy is adopted, to ensure than the consequences, specific to the altered system, are beneficial.

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APPENDIX 1

DETERMINATION OF AMMONIA

Introduction

The following method, taken from Parsons et al. (1984), was used to determine the total ammonia concentration in the recirculation system water.

A. Capabilities

Range: 0.1 to 10 μg-at l^{-1}

Precision at the 1 μg-at l^{-1} level: the correct value lies in the range, mean of n determinations +/- 0.1 $n^{-0.5}$ μg-at l^{-1}

B. Outline of method

Seawater is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside which acts as a catalyst. The blue indophenol colour formed with ammonia is measured spectrophotometrically.

C. Special apparatus and equipment

125 ml Erlenmeyer flasks. These must be cleaned with dichromate acid and rinsed thoroughly with distilled water. Automatic pipettes to dispense reagents.

D. Sampling procedure and storage

Temporary storage of seawater prior to analysis appears satisfactory in glass or polyethylene bottles, but analysis should not be delayed for more than 1-2 h at most. If analyses cannot be performed in this time period, samples should either be frozen at -15 ^{o}C or stored unfrozen in the presence of 2 ml of phenol solution per 50 ml of sample (Reagent 2, Section E). Samples may be stored in either manner for up to 2 weeks.

E. Special reagents

1. Deionized water

Distilled water is passed through a cation exchange column in the hydrogen form (30 cm long, 1-2 cm wide). This water should be prepared fresh for use.

2. Phenol solution

Dissolve 20 g of analytical grade phenol in 200 ml of 95 % v/v ethyl alcohol.

3. Sodium nitroprusside solution

Dissolve 1.0 g of sodium nitroprusside, $Na_2[Fe(CN)_5NO].2H_2O$, in 200 ml of deionized water. Store in a dark glass bottle; the solution is stable for at least a month.

4. Alkaline reagent

Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide in 500 ml of deionized water. The solution is stable indefinitely.

5. Sodium hypochlorite solution

Use commercially available hypochlorite (eg., "Chlorox") which should be about 1.5 N. The solution decomposes slowly and should be checked periodically (Note a).

6. Oxidizing solution

Mix 100 ml of reagent 4 and 25 ml of reagent 5. Keep stoppered while not in use and prepare fresh every day.

F. Experimental procedure (Note b)

1. Add 50 ml of seawater to an Erlenmeyer flask from a 50 ml measuring cylinder. Add 2 ml of phenol solution, swirl to mix, and add in sequence 2 ml of nitroprusside and 5 ml of oxidizing solution (Note c); mix after each addition by swirling the flasks.

2. Allow the flasks to stand at room temperature (20-27 ^{o}C) for 1 h. The top of the flask should be covered with "parafilm" or "saranwrap" during this period. The colour is stable for ca. 24 h after the reaction period (Note d).

3. Read the extinction at 640 nm in a spectrophotometer using a 10 cm cell length.

4. Correct the measured extinction for the reagent blank and calculate ammonia-nitrogen from the expression:

$$\mu g\text{-at N } l^{-1} = F \times E$$

where E is the corrected extinction and F is the factor as determined in section H.

G. Determination of blank

Carry out the method exactly as described in Sections F1 to F3 above using 50 ml of deionized water. Blank extinctions should not exceed about 0.075 in a 10 cm cell.

H. Calibration

1. Prepare 50 ml of standard ammonia solution. Dissolve 0.100 g of analytical grade ammonium sulfate in 1000 ml of deionized water. Add 1 ml of chloroform as a preservative and store in a refrigerator. The solution is stable for many months if well stoppered.

$$1 \text{ ml} = 1.5 \text{ } \mu\text{g-at N}$$

Pipette 1 ml of this solution into a 500 ml measuring flask and make up to 500 ml with ammonium-free seawater (note e). The concentration is equivalent to 3 μg-at N l^{-1}. This secondary standard solution should be made up fresh before use.

2. Carry out the determination above, Sections F1 to F3. Correct the extinction for the reagent blank and calculate F as:

$$F = \frac{3.0}{E_S}$$

where E_S is the corrected extinction. The value of F should be about 6.5 and standardization should be run in triplicate.

Notes

(a) To check on the strength of hypochlorite, dissolve 12.5 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in 500 ml of water. Add a few crystals (ca. 2 g) of potassium iodide (KI) to about 50 ml of water in a small flask and pipette in 1.0 ml of hypochlorite solution. Add 5-10 drops of concentrated hydrochloric acid (HCl) and titrate the liberated iodine with the thiosulphate solution until no yellow colour remains. Discard the hypochlorite when less than 12 ml of thiosulphate is used.

(b) Great care is necessary in this method to reduce contamination from external sources. Plastic gloves should be worn over the hands and no use of ammonia for other analyses should be allowed in the same laboratory.

(c) Some seawater samples may require more than 5 ml of oxidizing reagent. However, the pH after addition of this reagent must not exceed ca. 9.8.

(d) Serious over development of the blue colour may occur in high light intensities. This requires flasks to be wrapped in aluminium foil or kept in the dark.

(e) Ammonium-free seawater is desirable in the calibration. If this is not available, then 1 l of seawater should be boiled after addition of 5 ml of 1N NaOH and the volume reduced by 0.7 l. The volume is replaced to 1 l with deionized water after neutralization with equivalent HCl and is filtered through a glass fibre filter. Recirculation system seawater was used.

Critique of method based on experience from turbot study

1. Section F2 states that the colour of the reaction is stable for 24 h. This was not confirmed in this study. Read samples, sealed with parafilm, left overnight for a period of approximately 13 h and then re-read were found to have absorbance readings of approximately 50 % of the initial readings. All samples were therefore read within 1 - 3 h of the mixing of reagents.

2. Section D indicates that fresh samples must be analysed within 2 h, or stored frozen or with phenol. However the thawing period of the samples was approximately 3 h after frozen storage, which was longer than the recommended standing time. Phenol was added to the samples in addition to freezing to aid preservation during the thawing period.

3. Note b states that no other ammonia analyses should be allowed in the same laboratory, to avoid cross-contamination. If other analyses may influence the results, then other samples within the same experiment may also contaminate the samples. It was not possible to avoid this possibility entirely, but parafilm was used to seal the Erlenmeyer flasks even after the reading of a sample. The concentrated stock bottle of standard ammonia was opened, and the dilute standard made up, in another room to that in which the analyses were conducted.

APPENDIX 2

DETERMINATION OF NITRITE

Introduction

The following method was used to determine the nitrite concentration in the recirculation system water. The method is taken from Parsons et al. (1984).

A. Capabilities

Range: 0.01-2.5 μg-at l^{-1}

Precision at the 1 μg-at l^{-1} level: the correct value lies in the range, mean of n determinations +/- 0.03 $n^{-0.5}$ μg-at l^{-1}.

B. Outline of method

The nitrite in seawater is allowed to react with sulfanilamide in an acid solution. The resulting diazo compound is reacted with N-(1-naphthyl)-ethylenediamine and forms a highly coloured azo dye.

C. Special apparatus and equipment

125 ml Erlenmeyer flasks

50 ml measuring cylinder

D. Sampling procedure and storage

Rinse glassware with samples before using; measure 50 ml of seawater from a measuring cylinder into a 125 ml Erlenmeyer flask; analyze within a few hours and

preferably immediately.

E. Special reagents

1. Sulfanilamide solution

Dissolve 5 g of sulfanilamide in a mixture of 50 ml of concentrated hydrochloric acid (specific gravity 1.18) and about 300 ml of distilled water. Dilute to 500 ml with water. The solution is stable for many months.

2. N-(1-naphthyl)-ethylenediamine dihydrochloride solution

Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or directly a strong brown colouration develops.

F. Experimental procedure

1. Add 1.0 ml of sulfanilamide solution from an automatic pipette to each 50 ml sample, mix and allow the reagent to react for more than 2 min but less than 10 min to assure a complete reaction.

2. Add 1.0 ml of naphthylethylenediamine reagent and mix immediately. Between 10 min and 2 h afterwards, measure the extinction of the solution in a 10 cm cuvette at a wavelength of 543 nm.

3. Correct the measured extinction for the reagent (and turbidity, if necessary) blank and calculate the nitrite concentration as:

$$\mu g\text{-at N } l^{-1} = \text{corrected extinction x F}$$

where F is determined as in Section H below.

G. Determination of blank

Carry out the method exactly as described in Section F, 1-2, using distilled water in place of seawater

H. Calibration

1. Standard nitrite

Anhydrous, analytical grade sodium nitrite, $NaNO_2$, should be dried at 110°C for 1 h and 0.345 g dissolved in 1000 ml of distilled water; store in a dark bottle with 1 ml of chloroform as a preservative. The solution is stable for several months.

$$1 \text{ ml} = 5 \mu g\text{-at N}$$

Dilute 10.0 ml of this solution to 1000 ml with distilled water and use the same day.

2. Procedure

Prepare three standards by pipetting 2.00 ml of dilute standard into each of three graduated 50 ml cylinders; make to volume of 50 ml with distilled water, mix and transfer to each of three Erlenmeyer flasks. Carry out steps 1 and 2 in Section F; correct the extinction for the reagent blank and calculate F from the expression:

$$F = \frac{2.00}{E_S}$$

where E_S is the mean extinction of three standards, corrected for the blank. The value of F is approximately

2 under conditions described above.

Critique of method based on experience from turbot study

1. Compared with the ammonia determination method, this method was found to be less sensitive to external contamination and procedural inaccuracies. Triplicate results were usually similar.

G. Determination of blank

Carry out the method exactly as described in Sections F1 to F3 above using 50 ml of deionized water. Blank extinctions should not exceed about 0.075 in a 10 cm cell.

H. Calibration

1. Prepare 50 ml of standard ammonia solution. Dissolve 0.100 g of analytical grade ammonium sulfate in 1000 ml of deionized water. Add 1 ml of chloroform as a preservative and store in a refrigerator. The solution is stable for many months if well stoppered.

$$1 \text{ ml} = 1.5 \text{ } \mu\text{g-at N}$$

Pipette 1 ml of this solution into a 500 ml measuring flask and make up to 500 ml with ammonium-free seawater (note e). The concentration is equivalent to 3 μg-at N l^{-1}. This secondary standard solution should be made up fresh before use.

2. Carry out the determination above, Sections F1 to F3. Correct the extinction for the reagent blank and calculate F as:

$$F = \frac{3.0}{E_s}$$

where E_s is the corrected extinction. The value of F should be about 6.5 and standardization should be run in triplicate.

Notes

(a) To check on the strength of hypochlorite, dissolve 12.5 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in 500 ml of water. Add a few crystals (ca. 2 g) of potassium iodide (KI) to about 50 ml of water in a small flask and pipette in 1.0 ml of hypochlorite solution. Add 5-10 drops of concentrated hydrochloric acid (HCl) and titrate the liberated iodine with the thiosulphate solution until no yellow colour remains. Discard the hypochlorite when less than 12 ml of thiosulphate is used.

(b) Great care is necessary in this method to reduce contamination from external sources. Plastic gloves should be worn over the hands and no use of ammonia for other analyses should be allowed in the same laboratory.

(c) Some seawater samples may require more than 5 ml of oxidizing reagent. However, the pH after addition of this reagent must not exceed ca. 9.8.

(d) Serious over development of the blue colour may occur in high light intensities. This requires flasks to be wrapped in aluminium foil or kept in the dark.

(e) Ammonium-free seawater is desirable in the calibration. If this is not available, then 1 l of seawater should be boiled after addition of 5 ml of 1N NaOH and the volume reduced by 0.7 l. The volume is replaced to 1 l with deionized water after neutralization with equivalent HCl and is filtered through a glass fibre filter. Recirculation system seawater was used.

Critique of method based on experience from turbot study

1. Section F2 states that the colour of the reaction is stable for 24 h. This was not confirmed in this study. Read samples, sealed with parafilm, left overnight for a period of approximately 13 h and then re-read were found to have absorbance readings of approximately 50 % of the initial readings. All samples were therefore read within 1 - 3 h of the mixing of reagents.

2. Section D indicates that fresh samples must be analysed within 2 h, or stored frozen or with phenol. However the thawing period of the samples was approximately 3 h after frozen storage, which was longer than the recommended standing time. Phenol was added to the samples in addition to freezing to aid preservation during the thawing period.

3. Note b states that no other ammonia analyses should be allowed in the same laboratory, to avoid cross-contamination. If other analyses may influence the results, then other samples within the same experiment may also contaminate the samples. It was not possible to avoid this possibility entirely, but parafilm was used to seal the Erlenmeyer flasks even after the reading of a sample. The concentrated stock bottle of standard ammonia was opened, and the dilute standard made up, in another room to that in which the analyses were conducted.

APPENDIX 2

DETERMINATION OF NITRITE

Introduction

The following method was used to determine the nitrite concentration in the recirculation system water. The method is taken from Parsons et al. (1984).

A. Capabilities

Range: 0.01-2.5 μg-at l^{-1}

Precision at the 1 μg-at l^{-1} level: the correct value lies in the range, mean of n determinations +/- 0.03 $n^{-0.5}$ μg-at l^{-1}.

B. Outline of method

The nitrite in seawater is allowed to react with sulfanilamide in an acid solution. The resulting diazo compound is reacted with N-(1-naphthyl)-ethylenediamine and forms a highly coloured azo dye.

C. Special apparatus and equipment

125 ml Erlenmeyer flasks

50 ml measuring cylinder

D. Sampling procedure and storage

Rinse glassware with samples before using; measure 50 ml of seawater from a measuring cylinder into a 125 ml Erlenmeyer flask; analyze within a few hours and

preferably immediately.

E. Special reagents

1. Sulfanilamide solution

Dissolve 5 g of sulfanilamide in a mixture of 50 ml of concentrated hydrochloric acid (specific gravity 1.18) and about 300 ml of distilled water. Dilute to 500 ml with water. The solution is stable for many months.

2. N-(1-naphthyl)-ethylenediamine dihydrochloride solution

Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or directly a strong brown colouration develops.

F. Experimental procedure

1. Add 1.0 ml of sulfanilamide solution from an automatic pipette to each 50 ml sample, mix and allow the reagent to react for more than 2 min but less than 10 min to assure a complete reaction.

2. Add 1.0 ml of naphthylethylenediamine reagent and mix immediately. Between 10 min and 2 h afterwards, measure the extinction of the solution in a 10 cm cuvette at a wavelength of 543 nm.

3. Correct the measured extinction for the reagent (and turbidity, if necessary) blank and calculate the nitrite concentration as:

$$\mu g\text{-at N } l^{-1} = \text{corrected extinction} \times F$$

where F is determined as in Section H below.

G. Determination of blank

Carry out the method exactly as described in Section F, 1-2, using distilled water in place of seawater

H. Calibration

1. Standard nitrite

Anhydrous, analytical grade sodium nitrite, $NaNO_2$, should be dried at 110°C for 1 h and 0.345 g dissolved in 1000 ml of distilled water; store in a dark bottle with 1 ml of chloroform as a preservative. The solution is stable for several months.

$$1 \text{ ml} = 5 \mu g\text{-at N}$$

Dilute 10.0 ml of this solution to 1000 ml with distilled water and use the same day.

2. Procedure

Prepare three standards by pipetting 2.00 ml of dilute standard into each of three graduated 50 ml cylinders; make to volume of 50 ml with distilled water, mix and transfer to each of three Erlenmeyer flasks. Carry out steps 1 and 2 in Section F; correct the extinction for the reagent blank and calculate F from the expression:

$$F = \frac{2.00}{E_S}$$

where E_S is the mean extinction of three standards, corrected for the blank. The value of F is approximately

2 under conditions described above.

Critique of method based on experience from turbot study

1. Compared with the ammonia determination method, this method was found to be less sensitive to external contamination and procedural inaccuracies. Triplicate results were usually similar.